

Assessment of Subarachnoid Contrast Medium Neurotoxicity from Changes in Rabbit Behaviour

With Special Reference to Prediction of Clinical
Postmyelographic Side-effects of Iohexol Compared
with Those of Metrizamide

Pavel Maly



Lund

Malmö 1983

ASSESSMENT OF SUBARACHNOID CONTRAST MEDIUM NEUROTOXICITY
FROM CHANGES IN RABBIT BEHAVIOUR
With Special Reference to Prediction of Clinical
Postmyelographic Side-effects of Iohexol Compared
with Those of Metrizamide

av

Pavel Maly

Leg.läk., Malmö

AKADEMISK AVHANDLING

som med vederbörligt tillstånd av Medicinska
Fakulteten vid Universitetet i Lund för av-
läggande av medicine doktorsexamen kommer att
offentligen försvaras i universitetskliniker-
nas aula, Allmänna Sjukhuset i Malmö, fredagen
den 16 december 1983, kl. 13.00.

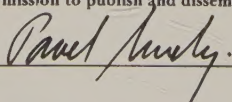
Organization LUND UNIVERSITY Department of Diagnostic Radiology Allmänna Sjukhuset S-214 01 Malmö Sweden	Document name DOCTORAL DISSERTATION	
	Date of issue 1983-12-16	
	CODEN: LUMEDW/MEXM-1001/1-144/1983	
Author(s) Pavel Maly	Sponsoring organization	
Title and subtitle Assessment of subarachnoid contrast medium neurotoxicity from changes in rabbit behaviour. With special reference to prediction of clinical postmyelographic side-effects of iohexol compared with those of metrizamide.		
Abstract The neurotoxic effect of the available monomeric non-ionic contrast media - metrizamide, iohexol, iopamidol, and iogluclide - was assessed, after their subarachnoid injection, by a method of observing and ranking excitative and depressive effects in the behaviour of albino rabbits. The ability of this method to detect signs of neurotoxicity was found to be as good as that of the visually evaluated EEG recordings. Iohexol exhibited the least severe neurotoxic effect of the four contrast media tested, and no convulsive or other excitative effect of this compound on the rabbit CNS was revealed. The neurotoxic effect of iopamidol and iogluclide was significantly more severe than that of iohexol, but significantly less severe than the neurotoxic effect of metrizamide that is currently used in clinical investigations of the subarachnoid space. Also, iohexol caused less severe alterations of the cation composition of the CSF (Na, K, Ca, and Mg) than metrizamide. A 14-day long intravenous premedication of rabbits with chlorpromazine significantly increased the convulsive effect of subarachnoidally injected metrizamide and the depressive effect of iohexol, whereas the convulsive effect of iohexol was still absent. The molecular toxicity of a contrast medium was found to be the decisive factor producing both types of changes in rabbit behaviour, whereas the volume or the osmolality of a contrast medium solution had no significant influence on the net neurotoxic effect of a contrast medium solution. The radiologic appearance of a subdural injection in rabbit was defined and the inadvertent subdural injection of a contrast medium solution was shown to cause significantly less severe changes in animal behaviour than the intended subarachnoid injection of the same contrast medium.		
Key words Adverse reactions, Behaviour, Chlorpromazine, Contrast media, CSF cations, Interaction, Neurotoxicity, Non-ionic, Osmolality, Rabbit, Subarachnoid space, Subdural space.		
Classification system and/or index terms (if any)		
Supplementary bibliographical information		Language English
ISSN and key title		ISBN
Recipient's notes	Number of pages 156	Price
	Security classification	

Distribution by (name and address)

Pavel Maly, Department of Diagnostic Radiology, Allmänna Sjukhuset, S-214 01 Malmö, Sweden

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature



Date October 17, 1983

From the Department of Diagnostic Radiology
(Head: Professor Tord Olin), University of Lund,
Allmänna Sjukhuset, S-214 01 Malmö, Sweden

ASSESSMENT OF SUBARACHNOID CONTRAST MEDIUM NEUROTOXICITY
FROM CHANGES IN RABBIT BEHAVIOUR
With Special Reference to Prediction of Clinical
Postmyelographic Side-effects of Iohexol Compared
with Those of Metrizamide

PAVEL MALY

MALMÖ 1983

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to all those who gave me their encouragement, indulgence, and ceaseless optimistic support during the course of this study, particularly my wife Eva and my closest relatives and friends. I especially want to thank

Docent Torsten Almén, my teacher and associate, who introduced me to his world of non-ionic contrast media, who gently moderated the excitative and depressive effects of this study on my CNS, and who, too, never failed in his efforts to teach me to think in a convergent way.

Professor Tord Olin for his encouraging support during this study;

Professor Erik Boijesen who allured me on the ways of investigating the unknown;

Docent Hans Olivecrona, one of my supervisors, for his calm criticism, collaboration, and repeated attempts to teach me to express myself in a "straight forward" manner;

Phil. Dr. Klaes Golman for his friendly collaboration, endless stimulating discussions, and indefatigable enthusiasm;

Cand. Pharm. Sigbjørn Salvesen who generously shared with us from his imposing experience in the field of experimental contrast media research;

Docent Dan Elmqvist and Docent Göran Fex whose interest and stimulating collaboration made parts of this study possible;

Dr. Curt G. Carlbom for his careful professional help in revising the English text.

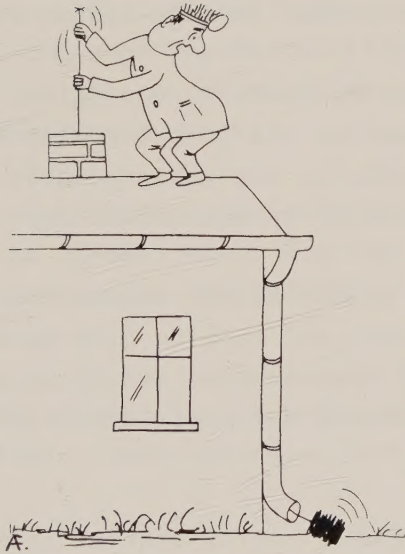
Mrs. Barbro Falkmer for excellent secretarial assistance as well as for her contagious encouraging optimism; Mrs. Britt-Marie Lilja, Mrs. Birgit Persson, and Mrs. Kerstin Thyberg for their skilful technical assistance and never-ceasing patience with both the rabbits and me.

Silent, but sincere thanks to the inventors of photocopying machines.

The investigation was supported by grants from The Swedish Medical Research Council, project Nos. 3483^{I-V} and 84^{II} and the Faculty of Medicine, University of Lund.



To Eva, Susanna, and Catarina



"... it may be stated that one cannot predict what paths future contrast medium research may take. Both expected and unexpected results are expected to occur in the future, and one cannot predict the unexpected."¹

This thesis is based on the following papers:

- I. Golman K, Olivecrona H, Gustafson C, Salvesen S, Almén T, and Maly P. Excitation and depression of non-anaesthetized rabbits following injection of contrast media into the subarachnoid space. Acta Radiol /Suppl/ (Stockh) 1980;362:83-86.
- II. Maly P, Elmqvist D, Almén T, and Golman K. Comparison between EEG and observation of rabbit behaviour in evaluation of subarachnoid neurotoxicity of metrizamide. Accepted for publication in Acta Radiol Diagnosis.
- III. Maly P, Olivecrona H, Almén T, and Golman K. Interaction between chlorpromazine and intrathecally injected non-ionic contrast media in non-anaesthetized rabbits. Submitted for publication in Neuroradiology.
- IV. Maly P, Almén T, Golman K, and Olivecrona H. Excitative effects in anaesthetized rabbits from subarachnoidally injected iso- and hyperosmolar solutions of iohexol and metrizamide. Submitted for publication in Neuroradiology.
- V. Maly P, and Fex G. CSF cation changes following subarachnoid injection of iohexol and metrizamide in rabbits. Accepted for publication in Acta Radiol Diagnosis.

These papers will be referred to in the text by their roman numerals, I-V.

ABBREVIATIONS AND DEFINITIONS

BBB = blood-brain barrier (includes both the blood-brain barrier and the blood-CSF barrier)

CNS = central nervous system

CSF = cerebrospinal fluid

ECOG = electrocorticography

EEG = electroencephalography

EMG = electromyography

ISF = interstitial fluid

In the present investigation, the terms neurotoxicity or neurotoxic effect of the contrast medium refer exclusively to the neurotoxic effect of the contrast medium after its administration into the subarachnoid space.

In the present investigation, the expressions significantly lower, higher, more severe, etc., mean exclusively that the difference is statistically significant - i.e. $p < 0.05$ or less.

The papers and references will be referred to in the text respectively by their roman numerals, I-V, and arabic numerals, 1-90.

CONTENTS

	Page
INTRODUCTION	7
AIM OF THE INVESTIGATION	12
MATERIAL AND METHODS	14
RESULTS	19
Deviations from Normal Animal Behaviour	19
Comparison of Two Methods - Evaluation of Behaviour and Evaluation of EEG	20
Comparison of Available Non-ionic Monomeric Contrast Media	21
Interaction in Neurotoxic Effects Between Contrast Media and Steroid Anaesthesia or Chlorpromazine.	22
Effect of Hyperosmolality and Large Volume of Contrast Medium Solution on Animal Behaviour	23
Changes of CSF Cations	24
Frequency and Radiographic Appearance of Subdural Injections of Contrast Medium	25
Difference in Behavioural Effects Between Subarachnoid and Subdural Injections of Contrast Medium	25
Differences in Subarachnoid Neurotoxicity and in Disturbing Effect on the CSF Cation Composition Between Iohexol and Metrizamide	26
DISCUSSION	29
SUMMARY AND CONCLUSIONS	43
REFERENCES	47
PAPERS I-V	

INTRODUCTION

Water-soluble contrast media for myelography should, for several reasons, be preferred to contrast media that are gases or oils.² In contrast to gas, a water-soluble contrast medium can produce a very high difference in attenuation between the subarachnoid space and adjacent structures. And in contrast to oil, a water-soluble medium fills the sleeves of nerve roots³ and is rapidly resorbed from the subarachnoid space.⁴ Rapid removal of a contrast medium from the subarachnoid space decreases the risk of arachnoiditis.

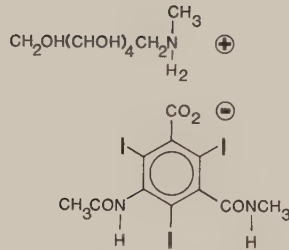
The high neurotoxicity of the first water-soluble contrast medium for myelography - sodium methiodal⁵ (1931) (Fig. 1a) - restricted its clinical use to lumbar myelography under the "protection" of spinal anaesthesia. It could not be used above the level of the conus medullaris, because it could cause convulsions in spite of spinal anaesthesia.^{6,7} Water-soluble contrast media (Fig. 1a) - meglumine iothalamate⁸ (1964) and its dimer meglumine iocarmate⁹ (1969) - that were developed later were less neurotoxic than sodium methiodal, and they could be used for lumbar myelography without spinal anaesthesia.¹⁰ When these media were administered above the level of the conus medullaris, they, too, frequently caused focal or generalized seizures,¹⁰⁻¹⁴ and therefore were not generally accepted for cervical myelography and cisternography.

Metrizamide^{15,16} (Fig. 1b) was the first non-ionic water-soluble contrast medium developed and was also the first water-soluble medium with a neurotoxicity that was so low that it became generally accepted for clinical use within the entire subarachnoid space including cervical myelography and cisternography.¹⁷ Metrizamide (Amipaque^R) was introduced for clinical use in 1972 and has since then been used in about 4 million myelographies.¹⁸

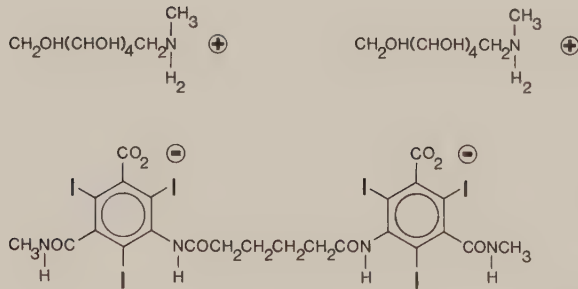
Ionic contrast media



ratio 0.5

Methiodal sodium

ratio 1.5

Meglumine iothalamate

ratio 2.0

Meglumine iocarmate

Fig. 1a. The chemical structures of water-soluble ionic contrast media used in the clinical investigations of the subarachnoid space.

Ratio = ratio between the number of iodine atoms and the number of osmotically active particles in an ideal solution of a contrast medium.

Non-ionic contrast media

ratio 3.0

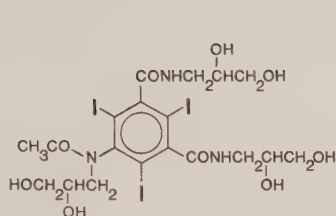
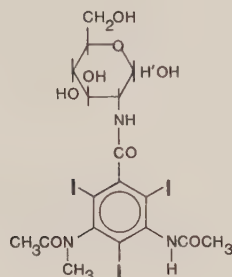
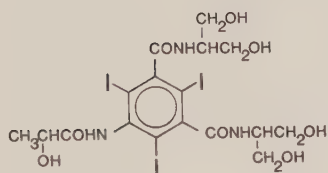
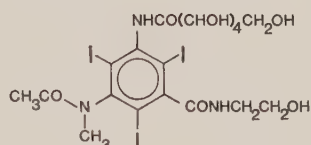
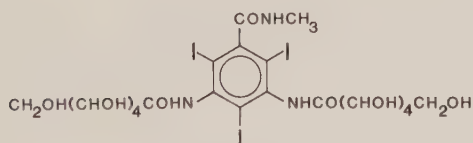
**Iohexol****Metrizamide****Iopamidol****Iogluclide****Ioglucomide**

Fig. 1b. The chemical structures of water-soluble monomeric non-ionic contrast media used or intended for use in the clinical investigations of the subarachnoid space. Ratio = ratio between the number of iodine atoms and the number of osmotically active particles in an ideal solution of a contrast medium.

The convulsive effect of metrizamide when injected into the subarachnoid space of animal or man is significantly lower than that of earlier employed water-soluble media,¹⁹⁻²⁶ and the reported clinical incidence of generalized seizures varies from 0.008 to 1.8 per cent of myelographies.^{27,28} Besides the rare convulsions, metrizamide also induces a variety of non-convulsive reactions. Side-effects such as headache, nausea, dizziness, vomiting, fever, leg or back pain occur in about 30-70 per cent of patients.^{2,26,27,29-32} There have also been some reports of more serious adverse reactions, such as perceptual aberrations or mental disturbances in the form of visual or auditory disturbances, various degrees of aphasia, reduced level of consciousness, confusion, hallucinations, and acute paranoic states.^{27,33-42} All of these reactions have been transient and have disappeared within a few hours, days, or weeks after the myelography.

The risk of side-effects increases with an increasing concentration or dose of metrizamide and with a more cephalad administration,^{28,32,37,43,44} and also possibly when it is used in patients that are taking drugs that lower the seizure threshold.^{45,46}

Metrizamide does not tolerate autoclaving and is therefore supplied as a lyophilized powder, with the attendant inconvenience that it has to be dissolved in water prior to use.

This chemical drawback of metrizamide and its induced non-convulsive side-effects in patients have resulted in the initiation of efforts to synthesize new water-soluble subarachnoid contrast media that will tolerate autoclaving and also will have at least, but preferably, an even lower neurotoxicity than metrizamide. Examples of such efforts are the non-ionic monomeric contrast media iopamidol⁴⁷ (1974), ioglunide⁴⁸ (1974), iohexol⁴⁹ (1976), and ioglucomide⁵⁰ (1981) (Fig. 1b). Dimeric non-ionic media have been synthesized during the

course of the present investigation and one of them, iotrol (1982),⁵¹ has been reported to have a lower neurotoxicity than metrizamide. The appearance of these new contrast media with possibly low neurotoxicity raises the question: To what extent can the occurrence of clinical side-effects following sub-arachnoid use of these media in man be predicted already from animal experiments?

Many experimental methods have dealt with the irritative and convulsive effects of subarachnoid media, but have disregarded the non-convulsive effects. In the case of metrizamide, its low convulsive effect in clinical use was correctly predicted from animal experiments,¹⁹⁻²⁶ but its numerous and diverse non-convulsive clinical side-effects had not been predicted from the preclinical animal experiments. Methods are therefore needed that will make it possible to predict both the convulsive and the non-convulsive clinical neurotoxicity of new sub-arachnoid media that are even less neurotoxic than metrizamide.

In the case of new subarachnoid contrast media with possibly lower neurotoxicity, larger doses of these media will be required in animal experiments to detect signs of the supposed low neurotoxicity. A large dose differs from a small clinical dose with regard to the three following parameters:

1. Number of contrast medium molecules injected into the cerebrospinal fluid (CSF).
2. Osmotic load placed on the CSF changing CSF osmolality.
3. Volume load placed on the CSF changing hydrostatic pressure of the CSF and changing the composition of CSF electrolytes, buffer, pH.

The effects of all of these "contrast medium parameters" in the CSF may also induce effects in the CNS. Therefore, the neurotoxic effects of a large subarachnoid contrast medium dose in an animal experiment might reflect poorly clinical

side-effects after a small clinical dose. For this reason, animal experimental methods are to be preferred that are sensitive enough to detect signs of neurotoxicity already at a small clinical dose.

If such sensitive methods do not become available, it becomes necessary to obtain knowledge about the neurotoxic effects induced by each of the previously mentioned parameters, i.e., number of contrast medium molecules, osmotic load, and volume load. A better understanding of each of these parameters of a contrast medium for subarachnoid use would (1) improve the possibility to predict from animal experiments the severity of side-effects to be expected from clinical use of new contrast media and (2) facilitate the selection of the best medium for testing in man.

AIM OF THE INVESTIGATION

The aim of the present investigation was to predict from subarachnoid injections in animals if any one of the available non-ionic monomeric contrast media induces side-effects that are so much less severe than those of metrizamide that it would be justified to test it clinically for myelography and cisternography in man.

Therefore it was decided:

to use a method that in non-anaesthetized animals could give information about both convulsive and non-convulsive effects on the CNS from subarachnoidally injected contrast media (paper I);

to determine which one of the available non-ionic monomeric contrast media causes the least severe side-effects after subarachnoid injection in rabbits (paper I).

In paper I, at the lower dose of the contrast medium, the method failed to discriminate differences between the least toxic media with regard to neurotoxicity. At a dose 10 times that of the normal clinical dose, iohexol manifested the lowest neurotoxicity among the compared contrast media (paper I). In order to avoid disturbing effects from such large volumes of contrast medium solutions and to be able to investigate the pure effects of contrast medium molecules, the research strategy was to design methods that would enable, at a low dose, the testing of contrast media less neurotoxic than metrizamide in the subarachnoid space (papers II and III). If this first strategy should fail, the second strategy was to investigate the neurotoxic effects from large volumes of the contrast medium solution in the subarachnoid space (papers IV and V).

From these strategies, it was decided:

to investigate whether visually evaluated EEG can detect signs of neurotoxicity induced by a subarachnoidally injected contrast medium at a lower dose than the method of observing animal behaviour (paper II);

to investigate whether a possible interaction between phenothiazine derivatives and non-ionic media can be utilized to increase differences in neurotoxicity between such media at a low (clinical) dose (paper III);

to investigate whether the osmolality of a contrast medium solution influences excitative behavioural effects induced by molecular toxicity of metrizamide and iohexol in rabbit (paper IV);

to investigate the possible changes of CSF cation composition that might be caused by subarachnoid injection of large volumes of iso-osmolal and hyperosmolal solutions of non-ionic contrast media in rabbit (paper V); and

to compare in rabbit the acute neurotoxicity of the new non-ionic contrast medium iohexol with that of metrizamide, which is currently used for clinical investigations of the subarachnoid space (papers I, III, IV, and V).

Because inadvertent subdural injections occurred after puncture of the cisterna magna (papers I and II), a decision was made:

to investigate whether subarachnoid and subdural injections of contrast media cause different changes in animal behaviour (paper III).

MATERIAL AND METHODS

A total of 324 albino rabbits (Swedish Land) of both sexes, which had free access to food and water, were included in the presented papers.^{I-V} Rabbits were excluded with blood-stained CSF after the suboccipital puncture or when the contrast medium was deposited into the subdural space. The excluded rabbits were immediately replaced.^{II,IV} Also, rabbits with inadvertent subdural deposition of the contrast medium were included in one investigation.^{III} None of the rabbits died after the contrast medium had been injected into the subarachnoid space. The body-weights of the rabbits ranged from 1.0 to 3.7 kg (median weights are given in the respective papers).

The method used in the present investigation included the following steps:

1. a) Injection of substances (contrast medium or a control substance) into the subarachnoid space through percutaneous needle puncture of the cisterna magna of either non-anaesthetized rabbits^{I,II,III} or anaesthetized rabbits;^{II,IV,V}

b) injection of additional drugs intravenously.^{II, III, IV, V} Substances injected intrathecally and additional drugs injected intravenously were administered at doses directly proportional to the body-weight of the rabbits (ml kg^{-1} or mg kg^{-1}).

2. Radiography to check the distribution of contrast media within the subarachnoid^{I-V} or the subdural space.^{III}
3. a) Detection and evaluation of the acute neurotoxic effects of substances injected into the subarachnoid^{I-IV} and the subdural^{III} space and/or of additional drugs injected intravenously;^{II, III, IV} (The neurotoxic effect was investigated during a 3-hour period immediately after the suboccipital injection and was evaluated by (1) observation and ranking of both excitative and depressive changes in animal behaviour,^{I, III} (2) observation of both excitative and depressive changes in animal behaviour and visual evaluation and scoring of EEG recordings from the same animals,^{II} and (3) observation and ranking of only the excitative changes in behaviour of anaesthetized animals.^{IV})
- b) sampling of the CSF before and 3 hours after the contrast medium injection and analysis of Na, K, Ca, Mg, and iodine concentrations in the samples.^V

The following non-ionic monomeric contrast media (ratio 3.0) were used in the present investigation:

Iohexol (Omnipaque^R) - Nyegaard & Co. A/S, Norway
 Metrizamide (Amipaque^R) - Nyegaard & Co. A/S, Norway
 Iopamidol (Iopamiro^R) - Bracco Industria, Italy
 Iogluclide (P297) - Laboratoire Guerbet, France

Their chemical structures are presented in Fig. 1 and their physical properties, at concentrations used in the present investigation, are tabulated in Table 1.

Table 1. Molecular weight and iodine content of non-ionic contrast media used. Concentration, viscosity, osmolality, buffering substance and its concentration, of contrast media solutions that were used in the present investigation

	Iohexol	Metriz- amide	Iopami- dol	Ioglunide (P297)
Molecular weight	821	789	777	807
Per cent iodine	46.4	48.2	49.0	47.2
Concentration used (mg I ml ⁻¹)	370	370	370	370
Viscosity 20°	29.6	37.0	18.0	30.0
(mPa s) 37°	13.5	16.0	9.5	13.0
Osmolality*				
(mmol l ⁻¹ H ₂ O)	980	580	800	800
Buffer**	Tris	NaHCO ₃	Tris	NaHCO ₃
(mmol l ⁻¹)	10.0	0.6	10.0	11.9
Concentration used (mg I ml ⁻¹)	144	166		
Osmolality* (mmol l ⁻¹ H ₂ O)		300	300	

* To be compared with the osmolality of rabbit CSF-305.2 mmol l⁻¹.⁵²

** To be compared with concentration of HCO₃ in rabbit CSF - 22 mmol l⁻¹.⁵²

SUMMARY OF MATERIALS

Table 2. Number of rabbits that received the contrast medium alone, control substance alone, contrast medium and an additional drug, and additional drug alone

Intrathecal injection				Intravenous injection						
Paper	Substance	Concentration (mg I ml ⁻¹)	Volume ₁ (ml kg ⁻¹ body-weight)	None	Alphaxolon/ Alphadolon	Chlorpro- mazine	Pento- barbital			
I	Metrizamide	370	0.5	15	-	-	-			
	Metrizamide	370	1.0	13	-	-	-			
	Iohexol	370	0.5	10	-	-	-			
	Iohexol	370	1.0	14	-	-	-			
	Iopamidol	370	0.5	13	-	-	-			
	Iopamidol	370	1.0	13	-	-	-			
	P297	370	1.0	14	-	-	-			
	0.9% NaCl	-	0.5	4	-	-	-			
	0.9% NaCl	-	1.0	4	-	-	-			
	Ringer	-	1.0	5	-	-	-			
II	none	-	-	-	10	-	-			
	Metrizamide	370	0.1	10	-	-	-			
	Metrizamide	370	0.1	-	10	-	-			
	Metrizamide	370	0.5	10	-	-	-			
	Metrizamide	370	0.5	-	10	-	-			
III	none	-	-	-	-	11	-			
	Metrizamide	370	1.0	12	-	-	-			
	Metrizamide*	370	1.0	4	-	-	-			
	Metrizamide	370	1.0	-	-	7	-			
	Metrizamide*	370	1.0	-	-	8	-			
	Iohexol	370	1.0	13	-	-	-			
	Iohexol*	370	1.0	4	-	-	-			
	Iohexol	370	1.0	-	-	8	-			
	Iohexol*	370	1.0	-	-	10	-			
	Iohexol*	370	1.0	-	-	-	-			
IV	none	-	-	-	-	-	7			
	Metrizamide	370	0.5	-	-	-	10			
	Metrizamide	166	1.11	-	-	-	10			
	Iohexol	370	0.5	-	-	-	15			
	Iohexol	144	1.28	-	-	-	10			
V	Metrizamide	370	0.5	-	-	-	9			
	Metrizamide	166	1.11	-	-	-	9			
	Iohexol	370	0.5	-	-	-	13			
	Iohexol	144	1.28	-	-	-	9			
Total = 324				158	+	30	+	44	+	92

* Subdural injection

The following control substances were injected into the sub-arachnoid space of rabbits:

Paper I. (Subarachnoid injection)

Unbuffered solution of NaCl 0.9% (ACO, Sweden): 0.5 and 1 ml kg^{-1} . Unbuffered Ringer solution (ACO, Sweden (147 mmol l^{-1} Na; 2.3 mmol l^{-1} Ca; 4 mmol l^{-1} K and 155.6 mmol l^{-1} Cl): 1 ml kg^{-1} .

The following additional drugs were administered intravenously to rabbits in the four other investigations (papers II-V):

Paper II. (Intravenous injection)

Alphaxolon/alphadolon (3/1) (Alphatesin^R, Glaxo Laboratories Ltd, England): 0.5 ml kg^{-1} .

Paper III. (Intravenous injection)

Chlorpromazine (Klorpromex^R, Dumex, Sweden): 5 mg kg^{-1} daily for 14 days.

Papers IV and V. (Intravenous injection)

Pentobarbital (Mebumal^R, ACO, Sweden): 30 ml kg^{-1} (initial dose).

The number of rabbits injected with the respective contrast medium, control substance, additional drug, or their combinations is given in Table 2.

Statistical Evaluation

The statistical analysis of the results in papers I-V was performed with the two-sided Wilcoxon rank sum test⁵³

($p < 0.05$ was considered to be a level of significance). Correlations between EEG patterns and scores for excitation and depression in paper II were assessed using Kendall's and Spearman's correlation coefficients.⁵⁴

RESULTS

Deviations from Normal Animal Behaviour

After subarachnoid and/or intravenous administration of certain substances or drugs, two types of deviations from normal behaviour were observed: (1) "excitative effects" - increased spontaneous neuromuscular activity or increased response to tactile and/or acoustical stimuli - and (2) "depressive effects" - decreased activity or response.

Excitative effects in animal behaviour^{I-IV} could be observed after subarachnoid injection of contrast media - metrizamide, iopamidol, and ioglunide (P297) - and after subarachnoid injection of more than 0.6 ml kg⁻¹ of 0.9% NaCl solution. No excitative effects^{I-IV} were observed after subarachnoid injection of iohexol, Ringer solution, 0.5 ml kg⁻¹ of 0.9% NaCl solution, and after intravenous administration of alphaxolon/alphadolon, pentobarbital, or chlorpromazine.

The severity of excitative effects increased in the following order:

1. Hyperexcitability, when a slight touching of the animal or a sudden noise evoked a muscular response of abnormal intensity.
2. Spontaneous tonic and/or clonic jerks with the head or the paws or local muscular twitchings. When the contrast medium could be presumed to accumulate on the right side of the subarachnoid space, the paw jerks occurred exclusively or predominantly on the contralateral side - left side.^{IV}
3. One generalized seizure or myoclonic jerks of the whole body.
4. More than one generalized seizure but recovery within 3 hours after injection of the contrast medium.
5. Many generalized seizures without recovery within 3 hours after the injection.

Among 13 rabbits that were injected with metrizamide and that displayed one or more generalized seizures or jerks of the whole body (excitative score 3-5), paroxysmal seizure activity occurred on the EEG of 12 rabbits.^{II} The chronological order of occurrence of the excitative effects in animal behaviour corresponded, as a rule, to the rank order of their severity; but in a few animals, paw jerks or a generalized seizure appeared prior to the occurrence of the signs of hyperexcitability. Every animal that, in the 3-hour period, displayed excitative signs of a definitely high degree also displayed signs of all lower degrees.

Depressive effects in animal behaviour could appear after subarachnoid injection of a hyperosmolal solution of all the contrast media used,^{I-III} as well as after intravenous administration of chlorpromazine.^{III} No depressive changes in animal behaviour were observed after subarachnoid injection of Ringer solution and 0.5 ml kg^{-1} of $0.9\% \text{ NaCl}$ ^I or after the recovery from alphaxolon/alphadolon anaesthesia.^{II}

The following degrees of depressive changes in animal behaviour were distinguished:

1. Uninterest in the other rabbits and difficulties and unwillingness to move around.
2. Inability to move around; the animal was lying pronely or on its side within 2 hours after the subarachnoid injection but it could move around within the third hour after the injection.
3. Inability to move around within 3 hours after the injection; the animal was lying pronely or on its side.

Comparison of Two Methods - Evaluation of Behaviour and Evaluation of EEG

The method of visually evaluated EEG did not detect metrizamide-induced signs of neurotoxicity at a lower dose level

than did the method of observing changes in animal behaviour.^{II} After subarachnoid injection of the clinical dose of metrizamide (0.1 ml kg^{-1} at 370 mg I ml^{-1}), signs of neurotoxicity (depressive changes) were observed in the behaviour of alphaxolon/alphadolon-anaesthetized rabbits that were significantly different from the normal behaviour of rabbits in the control group that were injected only with alphaxolon/alphadolon. In the same rabbits injected with metrizamide, the EEG could not detect signs of neurotoxicity that were significantly different from those in the control group.^{II} At the dose level of 0.5 ml kg^{-1} , both methods could detect significantly higher numbers of animals with more severe signs of neurotoxicity than at the lower dose level (0.1 ml kg^{-1}). There was a highly significant positive correlation between the degree of EEG abnormality and the degree of changes (both excitative and depressive) in animal behaviour.^{II} The maximum neurotoxic effect of metrizamide appeared in EEGs and animal behaviour within the pre-elected 3-hour period after metrizamide injection. After the end of this period, the metrizamide-induced neurotoxic effect decreased in both measured parameters (EEG and animal behaviour).^{II}

Comparison of Available Non-ionic Monomeric Contrast Media

Measured by ranking behavioural changes and taking into account both the excitative and the depressive changes, the four tested contrast media can be ranked^I according to their acute neurotoxicity after their subarachnoid administration in the following order: (1) iohexol, which caused significantly less severe signs of acute neurotoxicity than the remaining three contrast media; (2) ioglunide (P297) and iopamidol, both of which caused significantly less severe signs of neurotoxicity than metrizamide; and (3) metrizamide, which produced significantly more severe signs of acute neurotoxicity than the previously mentioned three contrast media.

The most distinctive difference in the neurotoxicity of these media was found in their excitative effects. Whereas iohexol did not produce any excitative effects, the other three contrast media produced excitative effects ranging from hyper-excitability to repeated generalized seizures.^I

Interaction in Neurotoxic Effects Between Contrast Media and Steroid Anaesthesia or Chlorpromazine

At both the clinical dose level (0.1 ml kg^{-1}) and at the level of 0.5 ml kg^{-1} of metrizamide 370 mg I ml^{-1} , there were more abnormal EEG patterns and more severe behavioural changes in rabbits that had been anaesthetized with alphaxolon/alphadolon than in non-anaesthetized rabbits, but the differences were not statistically significant.^{II} No depressive changes were detected after recovery from alphaxolon/alphadolon anaesthesia in rabbits that were not injected with metrizamide (control group). When compared with the control group, the depressive changes after subarachnoid injection of 0.1 ml kg^{-1} of metrizamide were significantly more severe only in the anaesthetized rabbits, not in the non-anaesthetized rabbits.^{II} This finding indicates a possibility of a weak interaction between alphaxolon/alphadolon anaesthesia and subarachnoid injection of metrizamide as regards the depressive behavioural effects.^{II}

Chlorpromazine significantly increased the severity of the excitative effects of metrizamide from a median score of 3 (one generalized seizure) to a median score of 5 (repeated generalized seizures without recovery within 3 hours).^{III} Chlorpromazine also increased the depressive effects of metrizamide, but the depressive effects were already so severe in rabbits that were not treated with chlorpromazine that the difference compared with the maximum depressive effects in chlorpromazine-treated rabbits was not significant.^{III}

Chlorpromazine did not increase any excitative effects of iohexol, and iohexol produced no excitative effect in any rabbit.^{III} However, chlorpromazine significantly increased the severity of the weak depressive effects of a hyperosmolal solution of iohexol from a median score of 0 to a median score of 3.^{III}

Effect of Hyperosmolality or a Large Volume of Contrast Medium Solution on Animal Behaviour

The net neurotoxic effect of a subarachnoid injection of a contrast medium solution was exclusively dependent on the chemical structure of the contrast medium and not on the volume or on the osmolality of the solution.^{I,IV} Iso-osmolal 0.9% NaCl solution caused severe generalized convulsions at a dose between 0.6 and 0.9 ml kg⁻¹, whereas iso-osmolal Ringer solution did not cause any changes in animal behaviour at a dose of 1.0 ml kg⁻¹.^I A volume of 1.28 ml kg⁻¹ of iso-osmolal iohexol solution, when injected into the subarachnoid space of rabbits under pentobarbital anaesthesia, did not cause any signs of hyperexcitability.^{IV}

Hyperosmolality or iso-osmolality of the solution that contained an equimolal dose of metrizamide had no significant influence on the excitative effects produced by metrizamide molecules.^{IV} A dose of 1.0 ml kg⁻¹ of iohexol 370 mg I ml⁻¹ - with an osmolality 3.2 times higher than that of CSF - neither caused any excitative effects in 27 of 27 non-anaesthetized non-premedicated rabbits nor any depressive effects in 20 of the 27 rabbits.^{I,III} Under the same experimental conditions, metrizamide - with an osmolality only 1.9 times higher than that of the CSF - produced significantly more frequent and severe excitative and depressive effects than iohexol; excitative effects were absent only in 8 of 25 rabbits and depressive effects were absent in 2 of 25 rabbits.^{I,III}

Changes of CSF Cations^V

An equimolal amount of iohexol and metrizamide was administered into the subarachnoid space of rabbits in four different solutions: Io hexol 144 mg I ml^{-1} (1.28 ml kg^{-1}) and metrizamide 166 mg I ml^{-1} (1.11 ml kg^{-1}), which are both iso-osmolal relative to the CSF; and io hexol 370 mg I ml^{-1} and metrizamide 370 mg I ml^{-1} (both 0.5 ml kg^{-1}), both of which are hyperosmolal relative to the CSF.

After a sudden dilution of the CSF due to injection of large volumes of contrast medium solutions, the concentrations of Na, K, Ca, and Mg increased either proportionally or non-proportionally to one another, depending on the kind of solution injected. The concentrations of Na and K increased proportionally to each other after injections of all four solutions and had reached about 80 per cent of their pre-injectional values 3 hours after the injection.

A uniform decrease that was proportionally the same for all four cations was noted only after injection of the iso-osmolal solution of io hexol. Three hours after injection of hyperosmolal io hexol, the concentration of Ca was significantly increased above concentrations of the three remaining cations, but the median concentration of Ca was not increased over its pre-injectional level. Following injection of both the iso-osmolal and the hyperosmolal metrizamide solutions, the median value of Ca concentrations was increased above its pre-injectional level, and the concentrations of Ca and Mg were significantly increased over concentrations of Na and K. After injection of hyperosmolal metrizamide solution, there was, in addition, a significant increase in the Ca concentration in relation to that of Mg.

Irrespective of differences in the volume (ranging from 0.5 to 1.28 ml kg^{-1}) and in the concentration (ranging from 144 to

370 mg I ml⁻¹) among injected solutions containing an equimolal dose of the contrast medium per kg body-weight, the median concentrations of iodine per group ranged between 15 and 18 mg I ml⁻¹ of the CSF sampled 3 hours after the contrast medium injection.

Hyperosmolal solutions of both iohexol and metrizamide caused a significantly higher inflow of water into the subarachnoid space (median values 13 and 8 per cent, respectively) than their respective iso-osmolal solutions (5 and 1 per cent).

Frequency and Radiographic Appearance of Subdural Injections of a Contrast Medium

A correct subarachnoid deposition of contrast medium after suboccipital injection could be verified only by radiography. Differences in the radiographic appearance of the subarachnoid and the subdural injections are described in paper III. In spite of a free flow of a clear fluid (CSF) before and of a clear fluid (CSF and contrast medium) after the suboccipital injection, the radiography disclosed that generally^{I-V} the suboccipital injection resulted in the intended subarachnoid deposition of contrast medium in about 80 per cent of the injections, whereas in about 20 per cent of the injections most or all the contrast medium was inadvertently deposited into the subdural space. In animals treated with chlorpromazine, the frequency of inadvertent subdural injections was as high as 53 per cent.^{III}

Difference in Behavioural Effects Between Subarachnoid and Subdural Injection of a Contrast Medium

The severity of the behavioural effects caused by suboccipital injection of both metrizamide and iohexol was significantly different depending on whether the contrast medium was injected into the subarachnoid space or inadvertently into the

subdural space.^{III} The most salient differences were (1) the total absence of excitative effects in chlorpromazine-treated rabbits in which metrizamide was deposited subdurally versus repeated generalized seizures in all chlorpromazine-treated rabbits in which metrizamide was injected subarachnoidally and (2) the significantly less severe depressive effects in chlorpromazine-treated rabbits injected with iohexol into the subdural space compared with the depressive effects caused by a subarachnoid injection of iohexol in chlorpromazine-treated rabbits^{III} (Fig. 2).

The depressive effects following subdural injection were most severe immediately after the injection, whereas the depressive effects of a subarachnoid injection occurred about 30 min after the injection.

Differences in Subarachnoid Neurotoxicity and in Disturbing Effect on the CSF Cation Composition Between Iohexol and Metrizamide

When measured by ranking behavioural changes, the neurotoxicity of iohexol was proved to be lower than that of metrizamide.^{I,III,IV} Iohexol did not cause any signs of excitation in any of the 71 rabbits injected subarachnoidally with this compound (Fig. 2), whereas under identical experimental conditions in 67 rabbits, metrizamide induced significantly ($p < 0.02$) more severe excitative effects ranging from hyperexcitability to repeated generalized seizures (Fig. 2).

^{I,III,IV} In non-anaesthetized rabbits that were not treated with chlorpromazine, iohexol exhibited a very weak depressive effect that was significantly ($p < 0.02$) lower than that of metrizamide (Fig. 2).^{I,III} In non-anaesthetized rabbits treated with chlorpromazine, iohexol exhibited a marked depressive effect that did not significantly differ from that of metrizamide (Fig. 2).^{III}

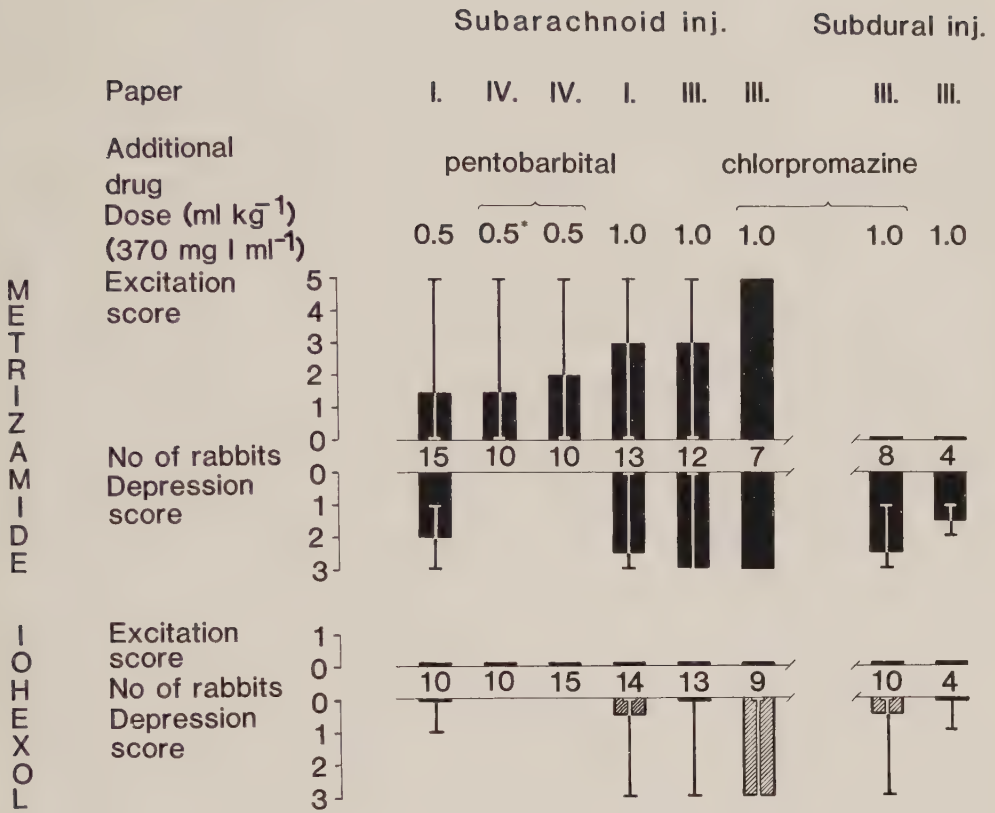


Fig. 2. Effects of metrizamide and iohexol on rabbit behaviour within 3 hours after injection of these contrast media into the subarachnoid or the subdural space. Scores of excitative and depressive effects (median and range).

Iohexol caused fewer changes than metrizamide in the proportions between the four main CSF cations Na, K, Ca, and Mg.^V No changes in proportions between cations were observed 3 hours after subarachnoid injection of the iso-osmolal iohexol solution; but after subarachnoid injection of the iso-osmolal metrizamide solution, the concentrations of Ca and Mg (expressed as a percentage of their pre-injectional values) were significantly higher than the concentrations of Na and K.^V

The hyperosmolal solution of iohexol (980 mmol l^{-1}) caused only a significant relative increase of Ca concentration as compared with the concentrations of Na, K, and Mg.^V The hyperosmolal solution of metrizamide (560 mmol l^{-1}) caused a significant relative increase of both Ca and Mg concentrations compared with those of Na and K, and also a significant relative increase of Ca concentration above that of Mg.^V

DISCUSSION

The choice of a contrast medium for clinical testing in the subarachnoid space of man, and with hopefully less severe side-effects than those of metrizamide, has to be made on the basis of animal experiments. In the present investigation, different contrast media were injected into the subarachnoid space of rabbits, and iohexol was found to be the compound that gave rise to the fewest and least severe changes in animal behaviour.^I Iohexol also caused less severe changes in the electrolyte composition of the rabbit CSF than metrizamide.^V The principal question is to what extent the differences between various contrast media, with regard to both the frequency and the severity of their clinical side-effects in man, can be predicted from animal experiments.

Some of the adverse reactions occurring after subarachnoid administration of contrast media, such as convulsions, fasciculations, hyperreflexia, and vomiting, are directly observable both in man and in animals and have thus an objective character. Several other adverse reactions, including headache, nausea, dizziness, and perceptual aberrations, have a subjective character and can be detected by interviewing the patient, which, of course, is unrealizable in animals. However, when such subjective adverse reactions reach a certain degree of severity, they may cause deviations from normal behaviour in both man and animals that are objectively observable, for example, decreased spontaneous locomotor activity during severe headache, nausea, or dizziness.

A new method for assessing acute contrast medium neurotoxicity by observing and ranking the deviations from normal behaviour of non-anaesthetized rabbits was used in the present investigation. The method, based on the suggestion of S. Salvesen,^I has been designed to evaluate differences between the possible future clinical adverse reactions following subarachnoid in-

jection of various contrast media in man by evaluating differences in the objective manifestations of such reactions as they occur in rabbits.

A particular advantage of this method is that the effects of contrast media can be studied on non-anaesthetized animals, I,II,III which conforms with most clinical investigations of the subarachnoid space in man. Anaesthesia, which is required in many animal investigations of contrast medium neurotoxicity that employ neurophysiologic electronic techniques - such as EEG, 22,24,55-58 ECoG, 21,59-61 EMG, 19,22,59,62 measuring of evoked corticospinal responses, 63-66 or segmental spinal reflexes 64,65,67 - may influence the detected effects of contrast media either by increasing or decreasing them. Further, a continuous anaesthesia precludes the registration of those contrast medium effects that change the degree of consciousness or otherwise decrease the locomotor activity of the animals. Also a short-lasting anaesthesia can increase depressive effects of contrast media that are observed in animals that have recovered from anaesthesia,^{II} and the effects can, in this way, be erroneously overestimated.

In the present investigation, the observed signs of the adverse reactions have been divided into two main groups depending on whether they increased or decreased the neuromuscular and locomotor activity of the animals and were named "excitative" and "depressive" changes in animal behaviour, respectively. The excitative and depressive changes could appear independently of each other, and some rabbits displayed only one of the two types of behavioural changes.

For example, hyperexcitability (excitation score 1) appeared in some rabbits injected with iopamidol, iogluclide, and metrizamide, and in some of these rabbits no signs of behavioural depression were detected.^{I,II,III} The hyperexcitability may be regarded as a lower degree of the same effect that causes the

spontaneous jerks in the paws and head and generalized seizures, because the hyperexcitability appeared in all rabbits that exhibited the latter excitative changes. The higher degree of excitative effect of metrizamide, iopamidol, and iogluclide - jerks in the paws (excitation score 2) - may be, in agreement with observations of Skälpe¹⁹ and Oftedal²⁴, regarded as the manifestation of a focal convulsive effect of these contrast media on the brain - which is supported by results of paper IV - rather than some local "irritative" effect on the spinal cord or nerve roots. The latter effect has been observed after lumbar subarachnoid administration of ionic contrast media (meglumine iothalamate).^{10,68} The highest degree of an excitative effect of contrast media on the rabbit CNS is, as in man, the generalized seizures.

The ability of the behavioural observation method to detect the excitative and convulsive effect of a contrast medium injected into the subarachnoid space of rabbits was as good as the method of the visually evaluated EEG.^{II} Also the severity of the excitative and convulsive effect, as expressed by the excitative scores on the rank scale, was assessed to be in a very good agreement with the severity of EEG changes and a highly significant ($p < 0.001$) positive correlation ($r = 0.88$ and $r = 0.91$) was found between excitation scores and patterns of EEG pathology.^{II}

It is difficult to determine what human clinical symptoms correspond to "the depressive effects of a contrast medium on the behaviour of non-anaesthetized rabbits". The aetiology of the depressive changes in experimental animals is much more heterogeneous than that of the excitative changes. Mild depressive changes (depressive score 1) were observed in 2 of 11 rabbits treated only with chlorpromazine, and thus these depressive changes must reflect only the sedative effect of this neuroleptic.^{III} Severe depressive changes were seen in chlorpromazine-treated rabbits in which metrizamide was inadver-

tently deposited in the subdural space and reached a very low, if any, concentration in the CNS.^{III} In these rabbits, the severe depression of their locomotor activity - median depression score 2.5 - might have been elicited by pain caused by the contact of a large amount of hyperosmolal solution with a large surface of the dura mater. Because the depressive effects following subdural deposition of contrast medium were most severe immediately after the injection,^{III} one of the possible mechanisms causing the depressive changes in the behaviour of these rabbits also might be the more than nine-fold increase of the intracranial CSF pressure that had been registered when identical doses of metrizamide had been injected subdurally into rabbits in another experiment.⁶⁹ Also following the correct subarachnoid injection of large volumes of hyperosmolal contrast media, the intracranial CSF pressure was increased by about 50 per cent⁶⁹ during the entire observation period and may thus contribute to the detected depressive effects. In rabbits that had repeated generalized seizures,^{I-III} the depressive changes might, at least in part, represent signs of postictal exhaustion. In some individual animals, signs of depression could be due to the complications of the puncture technique - such as a possible subarachnoid haemorrhage after the puncture or possible postpunctural leakage of the hyperosmolal contrast medium into the soft tissues of the neck through the puncture hole.

Besides these possible aetiologic mechanisms that may contribute to the net depressive effect of the subarachnoid injection of contrast media in the animals, it is tempting to assume that the depressive changes in animal behaviour mainly reflect the tendency of the contrast medium to produce, in animals, such non-convulsive side-effects as headache, nausea, dizziness, etc. The frequency of depressive changes in the behaviour of rabbits injected with the "clinical dose" of metrizamide (60 per cent of the rabbits)^{II} accords with the clinical frequency of non-convulsive adverse reactions -

headache, nausea, vomiting, dizziness, etc. - after subarachnoid administration of metrizamide in man that generally ranges between about 30 and 70 per cent.^{2,26,27,29-32} This agreement might suggest that the evaluation of depressive changes in animal behaviour after a "clinical dose" of metrizamide provides information about the frequency of the untoward side-effects in man after a clinical dose.

At the present time, the clinical side-effects of iohexol are being compared with those of metrizamide in lumbar myelography in man. When differences in the clinical non-convulsive adverse reactions between these contrast media are known from the clinical trials, it will be possible to determine more exactly to what extent the depressive changes observed in rabbit behaviour reflect the summarized severity of non-convulsive adverse reactions in man.

To sum up, the aetiology of the depressive deviations from normal animal behaviour is much more heterogeneous than that of the excitative deviations. Therefore, the prediction of the non-convulsive adverse reactions in man that is based on the present animal experiments must be regarded with a higher degree of uncertainty than prediction of the excitative and convulsive effects.

In order to produce statistically significant ($p < 0.05$) differences in behavioural effects of the non-ionic monomeric contrast media in a limited number of animals, doses of these contrast media had to be administered that exceeded clinically intended doses up to ten times.^I The "clinical dose" of metrizamide - the most neurotoxic non-ionic contrast medium tested - when injected into the subarachnoid space of 20 rabbits^{II} did not induce any signs of hyperexcitability or convulsions. Iopamidol when administered at a fivefold clinical dose into the subarachnoid space of non-anaesthetized rabbits did not induce any excitative effects, but the repeated generalized

seizures occurred after administration of a tenfold clinical dose of this compound.^I

It was thus at the tenfold clinical dose level when the convulsive effect of iopamidol could be distinguished from the non-occurring convulsive effect of iohexol.^I The effect of such large contrast medium doses on the central nervous system, the blood-brain barrier, and the CSF composition and pressure might, in the experimental animals, inadequately reflect the effect of clinical doses of contrast medium on the CNS, BBB, and CSF of man.

It can be assumed, in general, that the response of the CNS and of its homeostatic mechanisms is not directly proportional to the magnitude of the injurious agent. The dependency of the CNS response on the increasing magnitude of the noxa may be, for instance, logarithmic and there may also exist thresholds in such a responsiveness. The non-linearity of the responsiveness of the CNS on the toxicity of the injected substance and, possibly, the existence of a threshold in the responsiveness was suggested from observations that a subarachnoid injection of 0.5 ml kg^{-1} of physiologic NaCl solution did not elicit any behavioural changes, whereas after an injection of 0.6 to 0.9 ml kg^{-1} of the same solution, generalized seizures promptly occurred while the injection still was in progress.^I

The capacities of mechanisms responsible for the homeostasis within the brain and the subarachnoid space, including ion-pumps located at the nerve cell membranes or at the BBB and the buffering capacity of the CSF, are limited.^{70,71} It may be theoretically possible that the homeostatic mechanisms can correct small changes in the chemical composition of the CSF, pH, and osmolality that may be induced by the clinical contrast medium doses, whereas they may fail in correcting such changes that are caused by the large experimental doses that replace almost all the CSF in the subarachnoid space. For

these reasons, the effects of the physical properties of large contrast medium doses, such as osmolality, volume, and buffering capacity, deserve particular attention when results obtained from animal experiments using large doses may serve as a basis for prediction of effects of clinical doses in man.

Theoretically, the net neurotoxic effect of large doses of contrast medium administered into the subarachnoid space may represent a composite of effects of (1) the molecular chemotoxicity of the compound and effects of various characteristics of its solution such as (2) hyperosmolality, (3) volume, and (4) buffering capacity.

(1) The molecular toxicity of the compound is the most important factor inducing the clinical adverse reactions, which is demonstrated by the following facts: Many types of adverse reactions can occur after clinical myelography performed with a metrizamide solution iso-osmolal to the CSF;^{37,41,46,72-74} the non-ionic monomeric contrast media iohexol, iopamidol, ioglunide, and ioglucomide, which all have a higher osmolality than metrizamide, induce less severe effects in animal experiments both as regards the behavioural changes^{I,III,IV,50,66,75,76} and as regards the changes of electrophysiologic parameters^{51,66,77}.

(2) Hyperosmolality alone of solutions injected into the subarachnoid space has been shown to act depressingly on the EMG,⁶² the evoked corticospinal responses,⁶⁶ the spinal root reflexes,⁶⁷ and the evoked extracellular field potentials in the rat hippocampus.⁷⁸ Presently, it is not known to what extent the depressive effects of the hyperosmolality on these electrophysiologic events can literally be applied to the depressive changes in animal behaviour. Our experiments suggest that the effect of hyperosmolality within the subarachnoid space on the behaviour of the rabbits is very weak or none, because the almost entire replacement of the CSF by

iohexol 370 mg I ml⁻¹, which is 3.2 times hyperosmolal relative to the CSF, did not cause any depressive or excitative effects in 20 of 27 non-premedicated rabbits.^{I,III}

From experiments using the above-mentioned electronic neuro-physiologic techniques, it has been suggested⁶⁶ that the net neurotoxic effect of a contrast medium is a sum of two opposing effects: an excitative effect due to the molecular toxicity and a depressive effect due to the hyperosmolality of the solution. It could, therefore, not be excluded that some hypothetical depressive effect of hyperosmolality of iohexol solution could decrease some possible excitative effect of iohexol molecules so that the latter could not be revealed in animal behaviour.^{I,III} To eliminate such a hypothetical interfering effect of hyperosmolality, iohexol was injected sub-arachnoidally in a solution iso-osmolal relative to the CSF.^{IV} The proved absence of any excitative effect of iohexol in iso-osmolal solution^{IV} suggests that either the hypothetical depressive effect of hyperosmolality is very low or none, or that the convulsive effect of iohexol is extremely low. Moreover, there was no significant difference between the marked excitative effects induced by iso-osmolal and hyperosmolal solutions of equimolal dose of metrizamide,^{IV} which further supports our opinion that the hypothetical depressive effect of hyperosmolality that might interfere with the excitative effect of contrast medium molecules is low or none.

(3) The volume of the solution injected into the subarachnoid space causes dilution of the CSF and an increase of the CSF pressure. Most likely, neither the dilution of the CSF nor the increase of the CSF pressure influences the CNS of the experimental animals to such a degree that some changes in animal behaviour could be observed. No deviations from normal behaviour were, for instance, observed in 20 of 27 non-premedicated rabbits injected with 1.0 ml kg⁻¹ of iohexol^{I,III} or in all 5 rabbits injected with 1.0 ml of Ringer solution.^I

(4) It has been shown that the pH within the subarachnoid space became relatively low due to CO_2 and other brain metabolites after various unbuffered solutions were administered into the subarachnoid space of experimental animals.⁷⁹ An analogous decrease of the CSF pH is to be expected when large volumes of contrast medium with a very low buffering capacity, such as metrizamide, replace a considerable part of the CSF. A decrease in CSF pH might be involved in the mechanisms altering the blood-brain barrier permeability.⁷¹

Results of the present investigation^V may turn the attention to a factor that has not been considered previously, but that may contribute to the net neurotoxic effect of a contrast medium especially when large doses of a contrast medium are administered. This factor is an altered CSF cation composition, which may follow a subarachnoid injection of a contrast medium.

Subarachnoid injection of a contrast medium causes a uniform dilution of CSF cations only immediately after the injection. Whether or not the concentrations of various CSF cations remain proportional to each other during their return to the normal level depends on the characteristics of the contrast medium solution administered - its chemotoxicity, osmolality and, perhaps, its buffering capacity - which was demonstrated in paper V.

Only the injection of iso-osmolal iohexol caused an almost uniform dilution of the four main CSF cations still 3 hours after the injection.^V The same molar dose of iohexol administered as a hyperosmolal solution caused a significant relative increase in Ca concentration compared with concentrations of Na, K, and Mg. Both iso-osmolal and hyperosmolal metrizamide caused an increase of CSF Ca concentration above its pre-injectional level, and the concentrations of both Ca and Mg were significantly higher than those of Na and K. Moreover,

when metrizamide was administered as a hyperosmolal solution, the relative concentration of CSF Ca became significantly higher than that of Mg.^V These changes of the CSF cation composition were believed to be compatible with results of an increased permeability of the BBB due to molecular toxicity; hyperosmolality; and a possible metabolic acidification of the CSF after injections of metrizamide, which practically lacks buffer.^V

Several previous investigations cited by Bradbury (pp. 391-393)⁷⁰ have demonstrated a strong depressive effect of an increased CSF concentration of Ca and a weak depressive effect of an increased CSF concentration of Mg on both the excitability of the electrophysiologic processes in the CNS and on the behaviour of various animals, effects such as sleep or stupor. In the present investigation, the increase of CSF concentrations of Ca and Mg in relation to Na and K was larger in rabbits injected with hyperosmolal metrizamide than in rabbits injected with hyperosmolal iohexol,^V and in accordance with this, hyperosmolal metrizamide also caused significantly more depressive effects in the rabbit behaviour than hyperosmolal iohexol.^{I,III} Therefore, the hypothesis is advanced that some of the depressive effects following subarachnoid injection of large doses of hyperosmolal metrizamide are mediated by the relative increase in CSF Ca and Mg with respect to Na and K. This hypothesis may be generally valid for depressive effects of any substance or solution that possesses the ability to open the blood-brain barrier for the passage of Ca and Mg along their concentration gradients from fluids surrounding the BBB into the CSF. In man, only the increased concentration of Ca could be involved in such a mechanism producing depressive effects, because, contrary to the rabbit, man has a concentration of Mg outside the BBB that is lower than in the CSF⁵².

In spite of the fact that doses of iohexol were given^{I,III}

that almost replace the entire volume of the CSF (believed to be about 1,5-2 ml)^{52,80}, no excitative effects and only very weak depressive effects were proved in non-anaesthetized rabbits that were not premedicated with any other drug. If another contrast medium with toxicity close to that of iohexol should become available, it might be impossible to determine, using evaluation of its effects in non-anaesthetized, non-premedicated animals, whether that compound is less or more neurotoxic than iohexol. A possible approach to increase the ability of this experimental method to discriminate between neurotoxic effects of various contrast media could be the augmentation of the contrast medium-induced behavioural effects. This could be achieved by pretreating the animals with drugs that are known to lower the seizure threshold or have some other potentiating effect on the neurotoxic effect of contrast media. Chlorpromazine and other derivatives of phenothiazine are known to potentiate the excitative effect of metrizamide - to increase the frequency of generalized seizures after the subarachnoid administration of metrizamide in man⁴⁵ and in animals^{55,56,81}.

Daily premedication of rabbits with chlorpromazine during a 14-day period significantly increased the excitative effects of subarachnoidally injected metrizamide to the maximum effect that can be measured with our method of observation of animal behaviour - repeated generalized seizures without recovery within 3 hours in all animals.^{III} After such chlorpromazine premedication, there were still no excitative effects in rabbits subarachnoidally injected with iohexol.^{III} Chlorpromazine treatment thus increased the difference in excitative effects between metrizamide and iohexol compared with the difference in non-premedicated animals.^{III} On the other hand, chlorpromazine treatment decreased the significant difference in depressive effects between metrizamide and iohexol that was demonstrated in animals that had not received any premedication.^{I,III} This difference became considerably reduced as

chlorpromazine increased both the marked depressive effect of metrizamide and the weak depressive effect of iohexol.^{III}

An "intrathecal" administration of any substance, which is made after a puncture of the subarachnoid space by needle, may result in an inadvertent extra-arachnoid deposition of the substance. In man, the frequency of inadvertent extra-arachnoid depositions of contrast medium has been reported to be as high as 10-11 per cent.^{82,83} However, in animal research, no attention has been given to inadvertent subdural injection as a possible source of error giving spurious results. A free flow of clear fluid both before and after the "intrathecal injection" of any substance is not proof of a correct subarachnoid deposition, because the flow of clear fluid may also be equally free both before and after a subdural deposition. Such was the case in all the rabbits in the present investigation^{I-V} in which the contrast medium was inadvertently injected into the subdural space. The frequency of inadvertent subdural injections instead of the intended subarachnoid injections was about 20 per cent (71 of 361) of all suboccipital injections performed in rabbits that were not premedicated with chlorpromazine.^{I-V} In the chlorpromazine-premedicated rabbits,^{III} 53 per cent (18 of 34) of the intrathecal injections resulted in an inadvertent subdural deposition of contrast medium. In the chlorpromazine-treated rabbits, metrizamide deposited into the subdural space did not cause any excitative effects, whereas metrizamide deposited into the subarachnoid space induced maximum excitative effect - repeated generalized seizures - in all the rabbits.^{III} Also iohexol, when deposited into the subdural space caused significantly less severe depressive changes in chlorpromazine-treated rabbits than when deposited into the subarachnoid space.^{III}

It is, above all, the total absence of excitative effects after subdural injection of metrizamide in chlorpromazine-treated rabbits^{III} that most strikingly has shown that no or

only a small number of metrizamide molecules reached the sensitive structures of the CNS. This observation may be regarded as additional functional evidence supporting the opinion^{84,85} that it is the arachnoid membrane, and not the inner surface of the dura mater, that constitutes the blood-CSF border of the blood-brain barrier. That the arachnoid membrane acts as a barrier against contrast medium molecules is also indicated by findings showing that iohexol is eliminated significantly more rapidly from the subdural space of the rabbit than from the subarachnoid space.⁸⁶

To be absolutely certain of a subarachnoid deposition of any substance, a contrast medium must be injected together with the substance and checked with radiography. In studies not using contrast media, the possibility of an inadvertent subdural injection must be borne in mind when unexpected or controversial results - e.g., bimodal distribution of measured data⁸⁷ - are obtained after intrathecal injection.

The main aim of the present investigation was to decide from the observed manifestations of adverse reactions in rabbits which of the presently available non-ionic monomeric contrast media should be proposed for further clinical trials in the subarachnoid space of man. Iohexol, iopamidol, and ioglundide caused significantly less severe changes in the behaviour of non-anaesthetized rabbits than the presently clinically used metrizamide.^I Iopamidol and ioglundide have been shown, both in paper I and in other animal experiments,^{66,88,89} to cause excitative effects and generalized seizures that belong to the most serious clinically adverse reactions after injection of a contrast medium in the subarachnoid space of man. Iopamidol and ioglundide were therefore omitted from further investigations that were concentrated on the investigation of the possible side-effects of iohexol and their comparison with the side-effects of metrizamide.

The effort exerted in the present investigation to reveal the side-effects of subarachnoidally injected iohexol can be demonstrated by comparison between clinical side-effects of metrizamide in myelography in man and excitative and depressive changes in behaviour of animals injected subarachnoidally with metrizamide in the present study.

Whereas generalized seizures occur in 0.008^{27} to 1.8^{28} per cent of clinical myelographies performed with metrizamide in man, in the present investigation, generalized seizures after subarachnoid injection of metrizamide occurred in 100,^{III} 62,^I 58,^{III} 30,^{IV} and 20 per cent^{IV} of the rabbits, depending on the experimental design. Under the same experimental conditions, neither seizures nor other signs of hyperexcitability occurred after subarachnoid injection of iohexol.

Whereas such non-convulsive adverse reactions as headache, nausea, vomiting, dizziness occur in 30^2 to 70 per cent^{26,28} of clinical myelographies with metrizamide, in the present investigation, the subarachnoid injection of metrizamide caused depressive effects in behaviour of 93^I and 92 per cent^{III} of non-anaesthetized non-premedicated rabbits. Under the same experimental conditions, iohexol caused depressive changes in only 21^I and 23 per cent^{III} of the rabbits. Premedication of the animals with chlorpromazine increased the depressive effects of both compounds and 100 per cent of the rabbits injected with metrizamide and 89 per cent of the rabbits injected with iohexol displayed depressive changes in their behaviour.^{III} However, also in the chlorpromazine-premedicated rabbits, the depressive effects were less severe after subarachnoid injection of iohexol than after that of metrizamide. Rabbits that received the maximum depression score of 3 after iohexol were lying in the prone position, whereas all the rabbits injected with metrizamide that received the same score of 3 were lying on their side, which may be regarded as a more severe depressive effect.^{III}

The proved lower acute subarachnoid neurotoxicity of iohexol in rabbits as compared with metrizamide^{I,III,IV} accords with results from intracisternal and intracerebral injections in rats⁹⁰ and from pericerebral injections in guinea pigs evaluated by quantitative computerized EEG analysis⁸⁹.

The very marked difference demonstrated in the present investigation^{I,II,IV} between the non-occurring excitative effect of iohexol and the pronounced convulsive effect of metrizamide in rabbits suggests that the excitative and convulsive effect of iohexol, when administered into the subarachnoid space of man, will be considerably lower than the already low clinical convulsive effect of metrizamide. Also the frequency and severity of the non-convulsive adverse reactions may be expected to be lower in the clinical use of iohexol compared with that of metrizamide.

If iohexol does not cause any side-effects that are unpredictable from animal experiments, such as psychotic reactions, its use instead of metrizamide should decrease risks of serious adverse reactions in clinical investigations of the subarachnoid space, and particularly of its cranial parts - such as in cervical myelography, cisternography, and ventriculography.

SUMMARY AND CONCLUSIONS

An animal model was designed that made it possible to compare the acute neurotoxicity of subarachnoidally administered contrast media as regards both their convulsive side-effects and other, non-convulsive side-effects.

The ability of this model to detect signs of acute subarachnoid neurotoxicity of a contrast medium was found to be at least as good as that of the visually evaluated EEG.

Among the available non-ionic monomeric contrast media - metrizamide, iopamidol, ioglunide, and iohexol - iohexol caused the fewest and least severe adverse reactions when injected into the subarachnoid space of non-anaesthetized rabbits. No excitative or convulsive effect of iohexol could be evinced in the present investigation. When injected into the subarachnoid space of the rabbit, iohexol causes fewer disturbances of the cation composition of the CSF than metrizamide.

Results of the present investigation prove that it is the chemotoxicity of the molecule, and not the hyperosmolality of the solution, that mainly determines signs of excitation of the CNS after subarachnoid administration of monomeric non-ionic contrast media.

A hypothesis is advanced suggesting that depressive effects of subarachnoidally injected contrast media on animal behaviour may be, in part, mediated by the increase in Ca and Mg concentrations in the CSF relative to those of Na and K. Such an increase may follow an increase in the blood-brain barrier permeability induced by effects of chemotoxicity of contrast medium molecules and by effects of hyperosmolality of the contrast medium solution.

Results of the present investigation suggest that the arachnoid membrane acts as a barrier to contrast media.

The following predictions with clinical relevancy are based on results obtained from the present animal experiments:

1. The risk of seizures and other untoward side-effects that are associated with clinical investigations of the subarachnoid space - and particularly its intracranial parts - may be reduced by using iohexol (Omnipaque^R) instead of metrizamide (Amipaque^R).

2. The risk of seizures associated with investigations of the subarachnoid space in patients in which a discontinuation of the treatment with phenothiazine derivatives is impossible or non-advisable should be considerably lower or eliminated by using iohexol (Omnipaque^R) instead of metrizamide (Amipaque^R).
3. A physiologic NaCl solution should not be injected into the subarachnoid space in large doses because it may induce convulsions.

The following conclusions relevant to further experimental research can be drawn from the present investigation:

1. To discriminate between neurotoxic effects of sub-arachnoidally injected monomeric non-ionic contrast media in experimental animals, doses of these media must be used that greatly exceed those intended for clinical use.
2. Characteristics of large volumes of contrast media solutions, other than their molecular toxicity, may induce effects on animal behaviour or changes in CNS electrophysiology that may interfere with the studied effects of contrast medium chemotoxicity. A better understanding of such interfering effects should improve the value of experimental methods to predict the occurrence of clinical side-effects of myelographic contrast media.
3. There is a need for animal experimental methods that are able to discriminate between neurotoxic effects of low-toxic contrast media already at the clinical dose level.

4. The discrimination between neurotoxic effects of low-toxic contrast media in animal experiments could be facilitated by medicating animals with drugs that potentiate the neurotoxic effect of contrast media.
5. CSF cation changes following subarachnoid injection of contrast media deserve attention because they may help to elucidate some of the mechanisms of the neurotoxic action of contrast media on the CNS.
6. Whenever a substance is injected intrathecally, attention should be directed to the possibility of obtaining spurious results owing to an inadvertent subdural injection instead of the intended subarachnoid injection.

REFERENCES

1. Almén T. Experience from 10 years of development of water-soluble non-ionic contrast media. Invest Radiol (Suppl) 1980;15:S 283-8.
2. Maravilla KR, Neuwelt EA, Diehl JT. Application of metrizamide in the radiographic evaluation of the neurologically diseased patient. Neurosurgery 1979;5:389-406.
3. Kieffer SA, Binet EF, Esquerre JV, Hantman RP, Gross CE. Contrast agents for myelography: clinical and radiological evaluation of Amipaque and Pantopaque. Radiology 1978;129:695-705.
4. Almén T, Golman K. Pharmacology and toxicology of some intrathecal contrast media. In: Sackett JF, Strother ChM, eds. New techniques in myelography. Hagerstown, Maryland: Harper & Row, 1979:7-24.
5. Arnell S, Lidström F. Myelography with Skiodan (Abrodil). Acta Radiol (Stockh) 1931;12:286-8.
6. Lindblom K. Lumbar myelography by abrodil. Acta Radiol 1963;27:1-7.
7. Ahlgren P, Prestholm J. Komplikationer ved myelografi med methiodalum. (In Danish). Nord Med 1969;82:1600-4.
8. Campbell RL, Campbell JA, Heimbürger RF, Kalsbeck JE, Mealey J. Ventriculography and myelography with absorbable radiopaque medium. Radiology 1964;82:286-9.
9. Gonsette R, André-Balisaux G. A new hydrosoluble and resorbable contrast material for myelography and ventricu-

lography. XII International Congress of Radiology, Tokyo, 1969.

10. Gonsette R. An experimental and clinical assessment of water-soluble contrast medium in neuroradiology. A new medium-Dimer-X. Clin Radiol 1971;22:44-56.
11. Prestholm J, Lester J. Complications of myelography with Conray Meglumin. Acta Radiol Diagnosis 1972;13:860-4.
12. Ahlgren P. Dimer-X. A new contrast medium for lumbar myelography without spinal anaesthesia. Acta Radiol Diagnosis 1972;13:753-61.
13. Irstam L. Side effects of water-soluble contrast media in lumbar myelography. Acta Radiol Diagnosis 1973;14:647-56.
14. Haase J, Jepsen BV, Bech H, Langeback E. Spinal fracture following radiculography using meglumine iothalamate (Conray). Neuroradiology 1973;6:65-70.
15. Almén T. Contrast agent design. Some aspects on the synthesis of water soluble contrast agents of low osmolality. J Theor Biol 1969;24:216-26.
16. Lindgren E, ed. Metrizamide. A non-ionic water-soluble contrast medium. Experimental and preliminary clinical investigations. Acta Radiol (Suppl) 1973;335.
17. Lindgren E, ed. Metrizamide-Amipaque. The non-ionic water-soluble contrast medium. Further clinical experience in neuroradiology. Acta Radiol (Suppl) 1977;355.
18. Renaa T. Nyegaard & Co. A/S, Oslo, Norway. (Personal communication)

19. Skälpe IO. Myelography with metrizamide, meglumine iothalamate and meglumine iocarmate. An experimental investigation in cats. Acta Radiol (Suppl) 1973;335:57-66.
20. Gonsette RE. Biologic tolerance of the central nervous system to metrizamide. Acta Radiol (Suppl) 1973;335:25-44.
21. Oftedal SI, Kaye K. Epileptogenic effect of water-soluble contrast media. An experimental investigation in rabbits. Acta Radiol (Suppl) 1973;335:45-56.
22. Oftedal SI. Toxicity of water-soluble contrast media injected suboccipitally in cats. Acta Radiol (Suppl) 1973;335:84-92.
23. Salvesen S. Suboccipital injections of metrizamide to anaesthetized and unanaesthetized rabbits. Acta Radiol (Suppl) 1973;335:93-101.
24. Oftedal SI. Intraventricular application of water-soluble contrast media in cats. Acta Radiol (Suppl) 1973;335:125-32.
25. Skälpe IO, Torvik A. Toxicity of metrizamide and meglumine iocarmate after suboccipital injection in rats. Acute and long-term effects. Acta Radiol (Suppl) 1973;335:143-52.
26. Skälpe IO. Adverse effects of water-soluble contrast media in myelography, cisternography and ventriculography. A review with special reference to metrizamide. Acta Radiol (Suppl) 1977;355:359-70.
27. Ahlgren P. Early and late side effects of water-soluble contrast media for myelography and cisternography: A short review. Invest Radiol (Suppl) 1980;15:S 264-6.

28. Sackett JF, Strother ChM, Quagliari ChE, Javid MJ, Levin AB, Duff TA. Metrizamide-CSF contrast medium. Radiology 1977;123:779-82.
29. Ahlgren P. Amipaque myelography. The side effects compared with Dimer X. Neuroradiology 1975;9:197-202.
30. Amundsen P. Metrizamide in cervical myelography. Survey and present state. Acta Radiol (Suppl) 1977;355:85-97.
31. Nickel AR, Salem JJ. Clinical experience in North America with metrizamide. Evaluation of 1 850 subarachnoid examinations. Acta Radiol (Suppl) 1977;355:409-16.
32. Pettersson H, Fitz CR, Harwood-Nash DCF, Armstrong E, Chuang SH. Adverse reactions to myelography with metrizamide in infants, children and adolescents. I: General and CNS effects. Acta Radiol Diagnosis 1982;23:323-9.
33. Sortland O, Lundervold A, Nesbakken R. Mental confusion and epileptic seizures following cervical myelography with metrizamide. Report of a case. Acta Radiol (Suppl) 1977;355:403-6.
34. Gonsette RE. Cervical myelography with metrizamide by suboccipital puncture. Acta Radiol (Suppl) 1977;355:121-6.
35. Ruml E, Pallua A, Poewe W. Ungewöhnliche neurologische Ausfälle nach subokzipitaler Applikation von Metrizamid. Akt Neurol 1979;6:267-73.
36. Bohutová J, Vojír R, Kolár J, Grepl J. Some unusual complications of myelography and lumbosacral radiculography. Diagn Imaging 1979;48:320-5.

37. Schmidt RC. Mental disorders after myelography with metrizamide and other water-soluble contrast media. *Neuroradiology* 1980;19:153-7.
38. Smith MS, Laguna JF. Confusion, dysphasia, and asterixis following metrizamide myelography. *Can J Neurol Sci* 1980; 7:309-10.
39. Gelmers HJ. Acute psycho-organic reactions after cervical myelography using metrizamide. *Neurosurgery* 1981;83:57-61.
40. Koppejan EH, Begeer N, Bosch DA, Vencken LM. Organic psychosyndrome correlated with high density of grey matter on CT following metrizamide cervical myelography. *Clin Neurol Neurosurg* 1981;83:63-6.
41. Bergvall U, Brismar T, Lying-Tunell U, Valdimarsson E. Confusion, myoclonus and speech arrest: Epileptic manifestations after metrizamide myelography. *Acta Neurol Scand* 1981;63:315-22.
42. Bertoni JM, Schwartzman RJ, Van Horn G, Partin J. Asterix and encephalopathy following metrizamide myelography: Investigations into possible mechanisms and review of the literature. *Ann Neurol* 1981;9:366-70.
43. Skälpe IO, Amundsen P. Thoracic and cervical myelography with metrizamide. *Radiology* 1975;116:101-6.
44. Eldevik OP. Side-effects and complications of myelography with water soluble contrast agents. An experimental and clinical study. Thesis, Oslo 1983.
45. Hindmarsh T, Grepe A, Widén L. Metrizamide-phenothiazine interaction. Report of a case with seizures following myelography. *Acta Radiol Diagnosis* 1975;16:129-34.

46. Standnes B, Oftedal S-I, Weber H. Effect of levomepromazine on EEG and on clinical side effects after lumbar myelography with metrizamide. *Acta Radiol Diagnosis* 1982;23:111-4.
47. Felder E, Pitre D. Swiss Pat. No. 16588, 1974.
48. Tilly G, Hardovin MJC, Lautrou J. Offenlegungsschrift No.2456685, 1974.
49. Nordal V, Holtermann H. Brit. Pat. No. 1548594, 1976.
50. Hopkins RM, Adams MD, Lau DHM, Creighton JM, Brooke Hoey G. Ioglucomide: a new nonionic myelographic agent. Pre-clinical studies. *Radiology* 1981;140:713-7.
51. Sovak A, Ranganathan R, Kerber C. A nonionic, isotonic dimer iotrol: a new contrast medium for the intrathecal space. A review of pharmacological evaluation. In: Amiel M, ed. *Contrast media in radiology*. Berlin Heidelberg New York: Springer-Verlag, 1982:123-8.
52. Davson H. *Physiology of the cerebrospinal fluid*. London: J&A Churchill, 1967.
53. Lehman EL. Nonparametrics. In: *Statistical methods based on ranks*. San Francisco: Holden-Day Inc., Mc Graw-Hill, 1975:18.
54. Siegel S. *Non-parametric statistics for the behavioral sciences*. San Francisco: Mc Graw-Hill, 1956.
55. Grepe A, Widén L. Neurotoxic effect of intracranial sub-arachnoid application of metrizamide and meglumine iocarmate. An experimental investigation in dogs in neurolept analgesia. *Acta Radiol (Suppl)* 1973;335:102-18.

56. Grepe A, Widén L. Effects of cisternal application of metrizamide. An experimental investigation in dogs in N₂O analgesia with and without Halothane. Acta Radiol (Suppl) 1973;335:119-24.
57. Wylie IG, Afshar F, Koeze TH. Results of the use of a new water-soluble contrast medium (metrizamide) in the posterior fossa of the baboon. Br J Radiol 1975;48:1007-12.
58. Spencer CP, Chrisman CL, Mayhew IG, Kaude JV. Acute neurotoxicity of iopamidol following subarachnoid application. Proceedings of the Association of University Radiologists, annual meeting 1980. Invest Radiol 1980;15:411.
59. Sawhney BB, Oftedal SI. Reactions to suboccipital injection of water-soluble contrast media in rabbits. Acta Radiol (Suppl) 1973;335:67-83.
60. Nishikawa M, Yonekawa Y. Intracisternal Dimer X: Toxicity and prophylaxis. Neuroradiology 1976;11:61-5.
61. Nishikawa M, Shimizu Y. Toxicity of metrizamide compared with that of meglumine iocarmate. Kitano Hosp J Med 1978;23:13-16.
62. Piwonka RW, Healey JF, Rosenberg FJ. Intrathecal tolerance of metrizamide in chloralose anesthetized cats. Invest Radiol 1976;11:182-6.
63. Oftedal SI, Sawhney BB. Effect of water-soluble contrast media on cortically evoked potentials in the cat. Acta Radiol (Suppl) 1973;335:133-42.
64. Allen WE, Van Gilder JC, Collins WF. Evaluation of the neurotoxicity of water-soluble myelographic contrast

agents by electrophysiological monitors. *Radiology* 1976;118:89-95.

65. Hilal SK, Dauth GW, Burger LC, Gilman S. Effect of isotonic contrast agents on spinal reflexes in the cat. *Radiology* 1977;122:149-55.
66. Hilal SK, Dauth GW, Hess KH, Gilman S. Development and evaluation of a new water-soluble iodinated myelographic contrast medium with markedly reduced convulsive effects. *Radiology* 1978;126:417-22.
67. Bryan RN, Dauth GW, Gilman S, Hilal SK. Effects of radiographic contrast agents on spinal cord physiology. *Invest Radiol* 1981;16:234-9.
68. Praestholm J. Water-soluble contrast medium myelography in the experimental animal. A preliminary report. *Acta Radiol Diagnosis* 1972;13:848-59.
69. Carlborg B, Maly P, Golman K, Almén T, Lindqvist C. Changes in the cerebrospinal fluid pressure after intrathecal injection of contrast media in rabbits. In: Amiel M, ed. *Contrast media in radiology*. Berlin Heidelberg New York: Springer-Verlag, 1982:159-63.
70. Bradbury M. *The concept of a blood-brain barrier*. Chichester, New York, Brisbane, Toronto: John Wiley & Sons, 1979.
71. Fishman RF. *Cerebrospinal fluid in diseases of the nervous system*. Philadelphia, London, Toronto: W.B. Saunders Company, 1980.
72. Buruma OJS, Hekster REM. Transient areflexia following thoraco-lumbar myelography with metrizamide. Report of a

- case. *Acta Radiol (Suppl)* 1977;355:371-2.
73. Hekster REM, Prins HJ, Pennings-Braun AGM. Lumbar myelography with metrizamide. *Acta Radiol (Suppl)* 1977;355:38-41.
74. Eldevik OP, Nakken KO, Haughton VM. The effect of dehydration on the side effects of metrizamide myelography. *Radiology* 1978;129:715-6.
75. Sovak M, Siefert HM, Ranganathan R. Combined methods for assessment of neurotoxicity: Testing of new nonionic radiographic media. *Invest Radiol (Suppl)* 1980;15:S 248-53.
76. Sovak M, Deutsch JA, Ranganathan R. Evaluation of intracranial contrast media by aversion conditioning in rats. *Invest Radiol* 1982;17:101-6.
77. Sovak M, Ranganathan R, Johnson M. Spectral analysis of lapine EEG: neurotoxicologic evaluation of new nonionic contrast media. *Invest Radiol* 1980;15:452-6.
78. Hershkowitz N, Bryan RN. Extracellular effects of radiographic contrast agents on rat hippocampus. *Proceedings of the Association of University Radiologists, annual meeting* 1981. *Invest Radiol* 1981;16:393.
79. Elliott KAC, Jasper HH. Physiological salt solutions for brain surgery. Studies of local pH and pial vessel reactions to buffered and unbuffered isotonic solutions. *J Neurosurg* 1949;6:140-52.
80. Davson H, Kleeman CR, Levin E. Quantitative studies of the passage of different substances out of the cerebrospinal fluid. *J Physiol (London)* 1962;161:126-42.

81. Gonsette RE, Brucher JM. Potentiation of Amipaque. Epileptogenic activity by neuroleptics. *Neuroradiology* 1977;14:27-30.
82. Jones MD, Newton TH. Inadvertent extra-arachnoid injections in myelography. *Radiology* 1963;80:818-22.
83. Larson GM, Schall GL, DiChiro G. The unsuccessful injection in cisternography: Incidence, cause and appearance. In: Harbert JC, ed. *Cisternography and Hydrocephalus*. A symposium. Springfield, Illinois: Charles C Thomas, 1972:153-71.
84. Waggener JD, Beggs J. The membranous covering of neural tissues: An electron microscopy study. *J Neuropathol Exp Neurol* 1967;26:412-26.
85. Oldendorf WH. Cerebrospinal fluid formation and circulation. *Progr Nucl Med* 1972;1:336-58.
86. Holtz E, Aulie Michelet A, Jacobsen T. Absorption after subarachnoid and subdural administration of iohexol, ^{51}Cr -EDTA and ^{125}I -albumin to rabbits. *AJNR* 1983;4:338-41.
87. Lankelma J, Lippens RJJ, Drenthe-Schonk A, Termond EFS, van der Kleijn E. Change in transfer rate of methotrexate from spinal fluid to plasma during intrathecal therapy in children and adults. *Clin Pharmacokinet* 1980;5:465-75.
88. Gonsette RE, Brucher JM. Neurotoxicity of novel water-soluble contrast media for intrathecal application. *Invest Radiol (Suppl)* 1980;15:S 254-9.
89. Gonsette RE. Immediate and delayed neurotoxicity of newly synthesized contrast media demonstrated by EEG sequential analysis. In: Amiel M, ed. *Contrast media in radiology*.

Berlin Heidelberg New York: Springer-Verlag, 1982:141-8.

90. Siefert HM, Press WR, Speck U. Tolerance to iohexol after intracisternal, intracerebral and intraarterial injection in the rat. Acta Radiol (Suppl) 1980;362:77-81.

FROM THE RESEARCH DEPARTMENT, NYEGAARD & CO. A/S, OSLO, NORWAY, AND THE DEPARTMENT OF EXPERIMENTAL RESEARCH, MALMÖ ALLMÄNNA SJUKHUS, S-214 01 MALMÖ, SWEDEN.

EXCITATION AND DEPRESSION OF NON-ANAESTHETIZED RABBITS FOLLOWING INJECTION OF CONTRAST MEDIA INTO THE SUBARACHNOID SPACE

KLAES GOLMAN, HANS OLIVECRONA, CHRISTINA GUSTAFSON, SIGBJÖRN SALVESEN,
TORSTEN ALMÉN and PAVEL MALÝ

The advantages of the non-ionic contrast medium metrizamide (HOLTERMANN 1973, SACKETT & STROTHER 1979) compared with the different ionic contrast media when applied to the subarachnoid space have prompted the synthesis of other non-ionic contrast media among which iopamidol (FELDER & PITRE 1974), P297 (TILLY et coll. 1974) and iohexol (NORDAL & HOLTERMANN 1976) have been selected with the intention of clinical application.

In most investigations of the effects on the nervous system of contrast medium in the subarachnoid space the animals have been anaesthetized. This makes an evaluation of the toxicity of the contrast medium difficult due to interaction between this medium and other drugs (GREPE & WIDÉN 1973, GONSETTE & BRUCHER 1977). The excitation or depression caused by the contrast medium in contact with the brain or spinal cord may be potentiated or suppressed by such interaction.

Therefore, the effect on the nervous system—as reflected by excitation or depression—of iohexol, iopamidol and P297 injected into the subarachnoid space of non-anaesthetized rabbits was investigated. The effect was also compared with that caused by metrizamide, which has been clinically used in the subarachnoid space for several years.

Material and Methods

A total of 106 albino rabbits (Swedish land) weighing 1.0 to 3.7 kg were used. The rabbits were fixed in a box and after suboccipital puncture the solution to be tested was infused into the cisterna magna by an infusion pump (0.4 ml/min, Sage-instruments). Before connecting the needle (Butterfly 25 gauge) to the pump its correct position was controlled by free flow of clear cerebrospinal fluid. Continuous recording of the infusion pressure was performed during the infusion using a pressure transducer.

The solutions examined were: metrizamide (Amipaque, 370 mg I/ml), iopamidol (Bracco Industria, Italy, 370 mg I/ml), iohexol (Nyegaard & Co. A/S, Norway, 370 mg I/ml), P297 (Laboratoire Guerbet, France, 370 mg I/ml), Ringer (ACO, Sweden) 147 mmol/l Na, 2.3 mmol/l Ca, 4 mmol/l K and 155.6 mmol/l Cl, and NaCl 0.9% (ACO, Sweden).

The chemical structures of the contrast media have been given by HAAVALDSEN (1980). Two dose levels were tested, 0.5 ml/kg animal and 1.0 ml/kg except for P297 and Ringer solution which were used only at the dose 1.0 ml/kg.

After the infusion the rabbit was held in a vertical position with the head down for one minute in order to direct the test solution towards the basal cisterns. Radiography of the skull and cervical spine was then

Table

Median scores (range within parentheses) in convulsive and depressive indices after subarachnoid injection of different non-ionic contrast media, 0.9% saline and Ringer solution at the low dose (0.5 ml/kg=185 mg I/kg) and the high dose (1.0 ml/kg=370 mg I/kg); n=number of animals

Substance	185 mg I/kg			370 mg I/kg		
	Convulsive index	Depressive index	n	Convulsive index	Depressive index	n
Metrizamide	1.5 (0-5)	1.9 (0-3)	15	2.8 (0-5)	2.5 (0-3)	13
Iopamidol	0.0 (0-0)	0.4 (0-2)	13	1.5 (0-4)	1.0 (0-3)	13
P297	—	—	—	1.0 (0-4)	1.5 (0-3)	14
Iohexol	0.0 (0-0)	0.2 (0-1)	10	0.0 (0-0)	0.4 (0-3)	14
0.9% NaCl	0.0 (0-0)	0.0 (0-0)	4	4.0 ?	?	5
Ringer	—	—	—	0.0 (0-0)	0.0 (0-0)	5

performed within 5 min and the subarachnoid position of the contrast medium was confirmed. The animals were then observed for 3 hours and their behaviour was continually recorded with regard to excitation and depression. The effect of the test solutions was given scores according to the following system:

Excitation

Score:

- 0 No effect.
- 1 Hyperexcitability: a slight touching of the animal or a sudden noise evoked a muscular response of abnormal intensity.
- 2 Spontaneous jerks with the head or local muscular twitching.
- 3 One general epileptic fit or muscular twitching of the whole body.
- 4 More than one epileptic fit, recovery within 3 h.
- 5 Many epileptic fits without recovery within 3 h.

Depression

Score:

- 0 No effect. A rabbit spontaneously moving around, with normal motility behaviour and normal interest in other rabbits.
- 1 Difficulties to move around; recovery within 2 h.
- 2 The animal is lying prone or lateral; unable to sit within 2 h.
- 3 The animal is lying prone or lateral; unable to sit within 3 h.

All evaluations of the animals according to the

score system were made by an observer who was kept unaware of the identity of the infused solution.

A two-sided Wilcoxon's test with correction for ties (LEHMAN 1975) was used for statistical evaluation of the results; $p < 0.05$ was considered significant.

Results

The median scores of excitation and depression of the solutions injected into the subarachnoid space are given in the Table and in the Figure.

The animals infused with 0.5 ml/kg of saline ($n=4$) and 1.0 ml/kg of Ringer solution ($n=5$) showed no reaction at all.

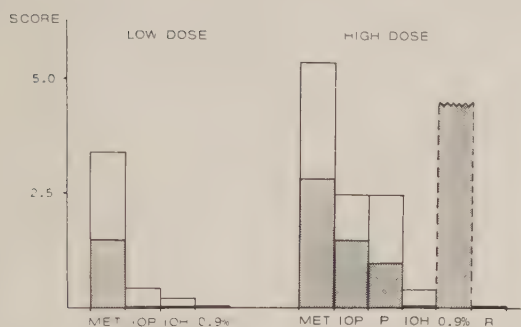
Saline in a dose of 1.0 ml/kg was intended to be given to 5 rabbits. These animals never received the whole dose because they got severe convulsions at doses between 0.6 and 0.9 ml/kg. The convulsions were so severe that continued infusion was made impossible and all these animals had to be killed within 30 min after the injection.

The median scores for the different contrast media and the reactions were ranked as follows (range within parentheses, n = number of animals):

Excitation (0.5 ml/kg)

Iohexol	0.0 (0-0), $n=10$
Iopamidol	0.0 (0-0), $n=13$
Metrizamide	1.5 (0-5), $n=15$

No difference was found between iohexol and iopamidol. They both caused less excitation than metrizamide ($p < 0.02$).



Median score in □ = excitation index and ▨ = depression index after subarachnoid injection of MET (metrizamide), IOP (iopamidol), P = (P297), IOH (iohexol) 0.9% (saline) and R (Ringer) at the low dose (0.5 ml/kg) and the high dose (1.0 ml/kg).

Depression (0.5 ml/kg)

Iohexol	0.2 (0-1), n=10
Iopamidol	0.4 (0-2), n=13
Metrizamide	1.9 (1-3), n=15

No significant difference between iohexol and iopamidol, both causing less depression than metrizamide ($p < 0.004$).

Excitation (1 ml/kg)

Iohexol	0.0 (0-0), n=14
P297	1.0 (0-4), n=14
Iopamidol	1.5 (0-4), n=13
Metrizamide	2.8 (0-5), n=13

Iohexol caused less excitation than any other medium ($p < 0.02$). No significant difference was found between P297 and iopamidol. P297 and iopamidol caused less excitation than metrizamide ($p < 0.05$).

Depression (1 ml/kg)

Iohexol	0.4 (0-3), n=14
Iopamidol	1.0 (0-3), n=13
P297	1.5 (0-3), n=14
Metrizamide	2.5 (0-3), n=13

No significant difference was observed between iohexol and iopamidol. Iohexol caused less depression than P297 and metrizamide ($p < 0.02$). No significant difference was found between iopamidol and P297. Iopamidol caused less depression than metrizamide ($p < 0.02$). The difference between P297 and metrizamide was non-significant.

Discussion

The most serious complications after injection into the subarachnoid space of ionic contrast media (methiodal, iothalamate, iocarmate) are general or focal convulsions. The introduction of the non-ionic medium metrizamide has markedly reduced the risk of convulsions in connection with clinical myelography (SKALPE 1977), whereas it has been less effective in reducing such adverse reactions as headache, nausea and vomiting.

The clinical finding that metrizamide caused less convulsions than other water-soluble contrast media had been predicted from animal experiments (SALVESEN 1977).

If the excitation score used in the present experiments reflects the tendency of a contrast medium to produce epileptic fits in man, the results indicate that the clinical use of iopamidol, P297 and especially of iohexol in the subarachnoid space should decrease the risk of epileptic seizures even below the risk with metrizamide. These results are in accordance with those of HILAL et coll. (1978), who in monkeys found that P297 caused less convulsions than metrizamide.

Saline produced severe convulsions when injected in volumes between 0.6 and 0.9 ml/kg. The fact that an equivalent volume of Ringer solution and a somewhat lower volume of saline (0.5 ml/kg) had no effect on the animals suggests that it was a fast disturbance of the ion balance, most likely the Na^+/K^+ ratio, which provoked the seizures. DUPREY & DROBECK (1975) injected metrizamide 300 mg I/ml or sodium chloride (1.4%) into the subarachnoid space in newborn rabbits. In accordance with the present findings doses of 0.835 ml/kg caused convulsions. The convulsions were more severe after injection of 1.4% NaCl than after a volume-equivalent dose of metrizamide. DUPREY & DROBECK also noted ataxia which may be compared with the depression seen in the present animals.

Whereas the excitation score noted may be taken as an indication of the probability of a contrast medium to produce epileptic fits in man, it is more speculative if the depression score predicts the occurrence of some other clinical adverse reactions, such as headache, nausea and vomiting. However, it is attractive to believe that some relation might exist between the experimentally noted depression score and the clinically observed dizziness and psychogenic depression (RICHERT et coll. 1979,

NICKEL & SALEM 1977) reported after myelography with metrizamide.

It was deliberately tried to direct as much as possible of the contrast medium into the cranial subarachnoid space, and although the location of the contrast medium was controlled at radiography of the skull it cannot be excluded that differences in viscosity between the media might have affected the amount of contrast medium reaching the cranial cavity. The cranial subarachnoid space must be considered as a very sensitive region with regard to contrast medium effects (WYLIE et coll. 1975), and it is possible that some spread of data within a single contrast medium group might be explained by this localization problem.

Conclusion

Iohexol injected into the subarachnoid space in rabbits without premedication caused less excitation than other media examined. Iohexol and iopamidol caused less depression than metrizamide. Physiologic saline without addition of other ions should not be injected subarachnoidally.

SUMMARY

The excitation and depression caused by four non-ionic contrast media (metrizamide, iopamidol, P297 and iohexol) have been examined after injection into the subarachnoid space of 0.5 and 1.0 ml/kg corresponding to 185 and 370 mg I/kg, respectively, in rabbits. Subjective blind evaluation was performed using a score index. Iohexol resulted in less and metrizamide in more excitation and depression than iopamidol and P297.

ACKNOWLEDGEMENT

This investigation was supported in part by the Swedish Medical Research Council (Project No. 3483). The authors would like to thank cand. real. Leiv Sandvik for statistical evaluation of the results and Mrs Britt-Maria Lilja for expert technical assistance.

REFERENCES

- DUPREY L. P. and DROBECK H. P.: Acute intracisternal toxicity of metrizamide in newborn (1-day old) and young adult rabbits. Internal report by Sterling-Winthrop Research Institute, 1975.
- FELDER E. and PITRE D.: Swiss Pat. No. 16588 (1974).
- GONSETTE R. E. and BRUCHER J. M.: Potentiation of Amipaque. Epileptogenic activity by neuroleptics. *Neuroradiology* 14 (1977), 27.
- GREPE A. and WIDÉN L.: Effects of cisternal application of metrizamide. An experimental investigation in dogs in N₂O analgesia with and without halothane. *Acta radiol.* (1973) Suppl. No. 335, p. 119.
- HAVALDSEN J.: Iohexol. Introduction. *Acta radiol.* (1980) Suppl. No. 362, p. 9.
- HILAL S. K., DAUTH G. W., HESS K. H. and GILMAN S.: Development and evaluation of a new water-soluble iodinated myelographic contrast medium with markedly reduced convulsive effects. *Radiology* 126 (1978), 417.
- HOLTERMANN H.: Introduction. *Acta radiol.* (1973) Suppl. No. 335, p. 1.
- LEHMAN E. L.: Nonparametrics. In: Statistical methods based on ranks, p. 18. Holden-Day, Inc., McGraw-Hill, San Francisco 1975.
- NICKEL A. R. and SALEM J. J.: Clinical experience in North America with metrizamide. Evaluation of 1850 subarachnoid examinations. *Acta radiol.* (1977) Suppl. No. 355, p. 409.
- NORDAL V. and HOLTERMANN H.: Brit. Pat. No. 1548594 (1976).
- RICHT S., SARTOR K. and HOLL B.: Subclinical organic psychosyndromes on intrathecal injection of metrizamide for lumbar myelography. *Neuroradiology* 18 (1979), 177.
- SACKETT J. F. and STROTHER C. M.: New techniques in myelography. Harper & Row, Publishers, Hagerstown, Maryland 1979.
- SALVESEN S.: Experimental investigation with metrizamide with relevance to the myelographic use. *Acta radiol.* (1977) Suppl. No. 355, p. 9.
- SKALPE I. O.: Adverse effects of water-soluble contrast media in myelography, cisternography and ventriculography. A review with special reference to metrizamide. *Acta radiol.* (1977) Suppl. No. 355, p. 359.
- TILLY G., HARDOVIN M. J.-C. and LAUTROU J.: Offenlegungsschrift No. 2456685 (1974).
- WYLIE I. G., AFSHAR F. and KOEZE T.: Results of the use of a new water-soluble contrast medium (metrizamide) in the posterior fossa of the baboon. *Brit. J. Radiol.* 48 (1975), 1007.

FROM THE DEPARTMENT OF DIAGNOSTIC RADIOLOGY, MALMÖ GENERAL HOSPITAL, S-214 01 MALMÖ, DEPARTMENT OF CLINICAL NEUROPHYSIOLOGY, UNIVERSITY HOSPITAL, S-221 85 LUND, SWEDEN, and RESEARCH DEPARTMENT, NYEGAARD & CO. A/S, OSLO 4, NORWAY

COMPARISON BETWEEN EEG AND OBSERVATION OF RABBIT
BEHAVIOUR IN EVALUATION OF SUBARACHNOID
NEUROTOXICITY OF METRIZAMIDE

P. MALY, D. ELMQVIST, T. ALMÉN and K. GOLMAN

Supported in part by the Swedish Medical Research Council
(Projects Nos. 84 and 3483).

SUMMARY

The non-ionic contrast medium metrizamide (370 mg I/ ml) was injected into the cisterna magna of rabbits at two dose levels (0.1 ml/kg and 0.5 ml/kg) to investigate whether visually evaluated EEG can detect neurotoxicity at a lower dose level than a method based on observation and ranking of signs of excitation and depression in animal behaviour. Half of the metrizamide injections were made without anaesthesia, whereas the remaining injections were made during a short alphaxolone/alphadolone anaesthesia. EEG and animal behaviour were followed for 24 h after the injection of metrizamide. At the low "clinical" dose, EEG could not detect statistically significant neurotoxicity, neither in anaesthetized nor in non-anaesthetized rabbits. Observation of animal behaviour, however, could detect statistically significant signs of depression in animals that had been anaesthetized. At the high dose level, both methods could detect significantly more serious symptoms of neurotoxicity than at the low dose level.

Introduction

The non-ionic contrast medium metrizamide is currently the most commonly used water-soluble medium in the subarachnoid space because of its lower convulsive effect than that of the earlier contrast media - methiodal, iothalamate, and iocarmate (AHLGREN 1975, 1980, SKALPE 1977). In about 30-70% of patients, metrizamide causes some minor adverse reactions, such as headache, nausea and vomiting; but it may occasionally cause such serious reactions as seizures, transitory mental disturbances, and ataxia (AHLGREN 1975, 1980, SKALPE 1977, AMUNDSEN 1977, NICKEL & SALEM 1977, MARAVILLA et coll. 1979, PETTERSSON et coll. 1982).

With the aim of decreasing such untoward reactions, new

compounds have been synthesized and tested in animals (HILAL et coll. 1978, SOVAK et coll. 1980, 1982, SPENCER et coll. 1980, HOLTZ et coll., GOLMAN et coll. 1980, GONSETTE & BRUCHER 1980, SIEFERT et coll. 1980). In a recent investigation (GOLMAN et coll. 1980) three new non-ionic media (P297, iopamidol, iohexol) and metrizamide were injected into the cisterna magna of non-anaesthetized rabbits and their excitative and depressive effects on animal behaviour were compared. To discriminate between the effects of the least toxic contrast media - iopamidol and iohexol - a tenfold increase of the "clinical" dose was required. Because of its large volume, such a dose changes the environment of the central nervous system (CNS). The large volume dilutes the cerebrospinal fluid (CSF) and changes its electrolyte concentration, osmolality, and buffering capacity. These effects are sources of error when efforts are made to predict the future clinical usefulness of new contrast media for the subarachnoid space from animal experiments.

A method that could detect differences in neurotoxicity between various contrast media at a clinical dose would eliminate such errors.

The first aim of the present investigation was to determine whether electro-encephalography (EEG) can even detect signs of contrast medium neurotoxicity at a clinical dose. Therefore, the effects of subarachnoid injection of metrizamide on EEG of non-anaesthetized rabbits were compared with those on rabbit behaviour to ascertain if EEG changes appear at a lower dose than changes in animal behaviour.

If anaesthesia is required during the suboccipital puncture of the subarachnoid space, a fast and short-acting intravenous anaesthetic should be used that does not interact with the contrast medium. The steroid anaesthetic alphaxolon/alphadolon is believed (GYERMEK & SOYKA 1975) to be such a compound. The second aim of the present investigation was to ascertain

whether or not there is an interaction between steroid anaesthesia and metrizamide.

Material and Methods

50 albino rabbits (Swedish Land), of both sexes and weighing 1.1-3.6 kg (median 1.9 kg), were used for the investigations. Rabbits were randomly allocated to 5 groups each comprising 10 animals. Animals with blood-stained CSF and/or a subdural deposition of metrizamide were excluded and replaced (totally 20 rabbits). EEG was conducted in all the rabbits before injecting any of the substances.

In group 1, no contrast medium was given and the rabbits were anaesthetized with a single intravenous injection of alphaxolon/alphadolon (3/1) (Alphatesin^R), 0.5 ml/kg body-weight. In group 2, the animals were injected with 0.1 ml/kg of metrizamide (Amipaque^R) (370 mg I/ml) into the cisterna magna. In group 3, they received both the anaesthetic and 0.1 ml/kg of metrizamide. In group 4, they were injected with metrizamide at a dose of 0.5 ml/kg; and in group 5, they were both anaesthetized and injected with 0.5 ml/kg of metrizamide (Table 1).

Suboccipital puncture of the cisterna magna was performed with a 25-gauge needle. When a free flow of clear CSF was obtained, the contrast medium was injected at a rate of 0.4 ml/min with an infusion pump. After injection of metrizamide, the rabbits were held with the head down for 1 min to direct the hyperbaric contrast medium into the intracranial subarachnoid space. Then, a lateral radiograph was taken and the distribution of the contrast medium was examined to exclude rabbits with a subdural injection. The rabbits were then placed in a 2 x 2-m cage. At 30-min intervals, their behaviour was observed for 5 min and, immediately afterwards, their EEGs

were recorded for 5 min.

In all the rabbits the combined observations of behaviour and the recording of EEG commenced at 30, 60, 90, 120, 150, 180 min, and 24 h after the injection of the anaesthetic in group 1 and after the injection of contrast medium in the other groups. In 24 rabbits from groups 2 to 5, these combined observations and recordings were, moreover, performed at 30-min intervals 3-13 h after the injection and in seven rabbits from groups 4 and 5 also once daily for 6 to 8 days after the injection.

EEG was recorded between two needle electrodes implanted symmetrically in the scalp 1.5 cm apart and 1.5 cm rostral to the ears. Between recordings, the electrodes were left in place but disconnected from the recorder, and the animal was left free in the cage. The EEG was recorded on a Siemens-Elema EEG-Mingograph (100 μ V/cm, time constant 0.3 s, upper frequency limit 30 Hz, and paper speed 30 mm/s).

All EEG records were visually evaluated by an experienced electro-encephalographer (D.E.) who had no knowledge about the contrast medium dose, anaesthesia, or animal behaviour.

Each 5 min recording was assigned to one of eight predetermined classes of EEG pathology according to the following schedule, in which a higher EEG class number indicates a more pathologic EEG.

EEG class	Dominant frequency	Paroxysmal activity
1	10-20	None
2	6-10	None
3	3-5	None
4	3-5	Spikes and sharp waves
5	1-3	None
6	1-3	Spikes and sharp waves
7	Variable and irregular	Periods of ictal activity lasting more than 3 seconds
8	Suppression of burst activity	

The EEG records obtained from one rabbit during a 3-h period after metrizamide injection and the record obtained 24 h after the injection were used to evaluate the neurotoxic effects of metrizamide in that animal, and each rabbit was then considered to belong to one of three EEG patterns, which are exemplified in Fig. 1. The three EEG patterns were designated 0, 1, and 2. Animals receiving a higher EEG-pattern number have had a correspondingly higher EEG-recorded pathology (higher EEG class numbers). The EEG-pattern is assumed to be an expression of the degree of neurotoxicity of the metrizamide injection. Criteria for assigning the EEG of an animal to one of these patterns are given in the following schedule.

EEG pattern

- 0 No or only insignificant EEG changes during the experiment. (All EEGs recorded between 30 and 180 min belonged to EEG class 1 or 2)

- 1 Moderate slowing in several recordings, but without obvious paroxysmal discharges. (All EEGs recorded between 30 and 180 min belonged to EEG classes 2 and 3)
- 2 Severe slowing and/or frequent paroxysmal discharges in several recordings. (EEG class 4 or higher in at least three of the recordings between 30-180 min).

If, for example, a rabbit had had one EEG recording belonging to class 5 and the remaining ones belonging to class 3 or below, it would have been assigned to EEG pattern 1. Similarly, if a rabbit had had one recording belonging to class 5, one to class 4, and the remaining ones to class 3 or below, it would have been assigned to EEG pattern 2. Such rabbits were not observed in this study.

Evaluation of changes in animal behaviour was made by an observer who did not have knowledge of the metrizamide dose nor have access to the EEG recordings. Signs of excitation and depression in animal behaviour were ranked according to a previously used system (GOLMAN et coll.):

Excitation

Score

- 0 No effect
- 1 Hyperexcitability: a slight touching of the animal or a sudden noise evoked a muscular response of abnormal intensity
- 2 Spontaneous jerks with the head or local muscular twitching
- 3 One generalized seizure or myoclonic jerks of the whole body
- 4 More than one generalized seizure, recovery within 3 h
- 5 Many seizures without recovery within 3 h

Depression

Score

- | | |
|---|---|
| 0 | No effect. A rabbit spontaneously moving around with normal motility behaviour and normal interest in other rabbits |
| 1 | Difficulties in moving around; recovery within 2 h |
| 2 | The animal lying pronely or on its side and unable to sit within 2 h |
| 3 | The animal lying pronely or on its side and unable to sit within 3 h |

The statistical analysis was performed with the two-sided Wilcoxon rank sum test ($P < 0.05$ was considered a level of significance). Correlations between EEG patterns and scores for excitation and depression were assessed using Kendall's and Spearman's correlation coefficients (SIEGEL 1956).

Results

After recovery from the anaesthesia, the rabbits in the control group (group 1), which had not been injected with metrizamide, had a normal EEG and their behaviour was normal (Table 1).

EEG did not detect metrizamide-induced signs of neurotoxicity at a lower dose than did the method of observing changes in animal behaviour (Tables 1 and 2). At the low metrizamide dose (0.1 ml/kg) - equivalent to the one used in clinical myelography - neither EEG nor observation of animal behaviour could detect significant neurotoxicity in non-anaesthetized animals (group 2). In animals that had been anaesthetized and injected with the same dose (0.1 ml/kg) of metrizamide (group 3), EEG was not significantly changed; but the method of observing changes in animal behaviour did detect neurotoxicity

Table 1

Number of non-anaesthetized rabbits or those that had been anaesthetized with alphaxolon/alphadolol with respective EEG pattern or score for changes in behaviour after subarachnoid injection of metrizamide 370 mg I/ml at two different dose levels (Group 1 is a control group)

Group	Dose: ml/kg		EEG pattern	Observation of Animals' Behaviour															No of animals
	Metrizamide	Alphaxolon/ /alphadolon		0	1	2	0	1	2	3	4	5	0	1	2	3			
1	-	0.5	10	-	-	-	10	-	-	-	-	-	10	-	-	-	10		
2	0.1	-	8	2	-	-	9	1	-	-	-	-	5	4	-	1	10		
3	0.1	0.5	7	3	-	-	7	3	-	-	-	-	3	5	1	1	10		
4	0.5	-	1	4	5	-	3	1	2	2	2	2	-	1	2	7	10		
5	0.5	0.5	-	-	10	-	-	1	1	1	4	3	-	-	3	7	10		

Table 2

Statistical analysis of differences in EEG patterns and scores of excitative and depressive effects caused by subarachnoid injection of metrizamide at two different dose levels in non-anaesthetized rabbits and those that had been anaesthetized with alphaxolon/alphadolon

Purpose of the comparison	Group with less changes		versus (v.)	Group with more changes		Probability for acceptance of equality between the groups compared*			
	Group Metriz. ml/kg	Anaesthesia		Group Metriz. ml/kg	Anaesthesia	EEG	Excitation	Depression	
Detection of neurotoxicity at "clinical dose" level	1	-	yes	2	0.1	no	NS	NS	NS
	1	-	yes	3	0.1	yes	NS	NS	P < 0.01
Dose effect of metrizamide	2	0.1	no	4	0.5	no	P < 0.01	P < 0.01	P < 0.01
	3	0.1	yes	5	0.5	yes	P < 0.01	P < 0.01	P < 0.01
Interaction with anaesthesia	2	0.1	no	3	0.1	yes	NS	NS	NS
	4	0.5	no	5	0.5	yes	NS	NS	NS

* Two-tailed Wilcoxon rank sum test. P > 0.05 considered not significant (NS) difference.

Table 3

Scatter plot: number of rabbits with identical combination of EEG pattern and behavioural change scores; and coefficients of correlation between EEG pattern and scores for excitation and depression

Excitation score	5	-	-	5	Depression score
	4	1*	-	5	
	3	-	-	3	3 1* 3 12
	2	-	1	1	2 - 3 3
	1	1	6	1	1 7 3 -
	0	24	2	-	0 18 - -
EEG pattern	0	1	2		EEG pattern 0 1 2
Correlation coefficients					Correlation coefficients
Kendall	$\tau = 0.906$	$(P < 0.001)$			Kendall $\tau = 0.915$ $(P < 0.001)$
Spearman	$r_s = 0.876$	$(P < 0.001)$			Spearman $r_s = 0.847$ $(P < 0.001)$

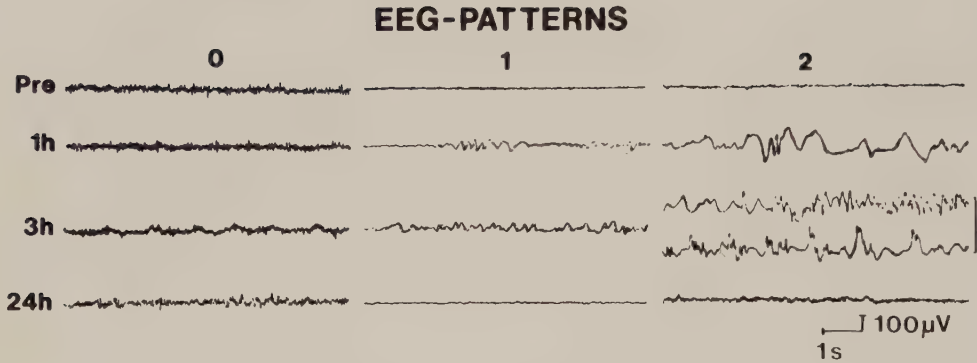


Fig. 1. Typical EEG recordings obtained before and after 1, 3, and 24 h after the subarachnoid injection of contrast media from rabbits assigned to EEG pattern 0, 1, and 2.

In the rabbit with EEG pattern 0, all four EEGs were normal and belonged to EEG class 1.

In the rabbit with EEG pattern 1, the EEGs at 1 and 3 h belonged to EEG class 3. The pre- and 24-h EEGs belonged to EEG class 1.

In the rabbit with EEG pattern 2, considerable slowing and paroxysmal discharges are apparent at 1 h (EEG class 6). At 3 h, the first recording shows pronounced slowing and the beginning of seizure activity. At the end of this recording, a clinical seizure was observed and the second recording obtained 70 seconds later shows the end of seizure activity (EEG class 7). The pre- and 24-h EEGs were normal (EEG class 1).

and signs of depression occurred at a significantly higher frequency in this group (group 3) than in the control group (group 1).

At the high metrizamide dose (0.5 ml/kg), both methods - EEG and observation of animal behaviour - could detect significantly higher numbers of animals with signs of neurotoxicity than at the low dose (0.1 ml/kg) in both non-anaesthetized (group 4) and previously anaesthetized animals (group 5). Nineteen of 20 animals in groups 4 and 5 had an abnormal EEG, and all of these 20 rabbits exhibited changes in behaviour, with signs of both excitation and depression. At the high dose (0.5 ml/kg), the signs of neurotoxicity were more serious than at the low dose (0.1 ml/kg); EEG revealed the highest degree of abnormality - "pattern 2" - in 15 of 20 animals and observation of animal behaviour revealed generalized seizures in 14 out of 20 animals.

A comparison between the two methods, in all 50 animals, gave a highly significant ($P < 0.001$) positive correlation between EEG abnormality and changes in animal behaviour (Table 3). All 15 rabbits with the highest degree of EEG abnormality (pattern 2) manifested abnormal behaviour, whereas only one of 26 rabbits with a normal EEG (pattern 0) displayed abnormal behaviour (Table 3, the rabbit indicated by *).

A maximum of neurotoxic effects appeared in EEGs and animal behaviour within the pre-elected 3-h period after the injection of metrizamide. The median time for the commencement of generalized seizures, observed in groups 4 and 5, was 90 min (range 30-180 min). Rabbits observed during the period 3-13 h after injection showed a decrease in metrizamide-induced neurotoxic effects both in their EEG recordings and in their behaviour; and during the period 24 h to 8 days after metrizamide injection, all had a normal EEG and displayed normal behaviour.

At both dose levels of metrizamide, there were more abnormal EEG patterns and more behavioural changes in previously anaesthetized than in non-anaesthetized animals (Table 1). These differences, however, were not significant (Table 2). The possibility of an interaction between metrizamide and alphaxolon/alphadolon is indicated by the finding that signs of behavioural depression in animals injected with the low metrizamide dose (0.1 ml/kg) were significantly higher than in the control group (group 1) only in the previously anaesthetized animals (group 3) but not in the non-anaesthetized rabbits (group 2) (Table 2).

Discussion

A dose of 0.1 ml/kg animal weight at a concentration of 370 mg I/ml corresponds to a currently used clinical dose employed in lumbar myelography - i.e., about 15 ml of metrizamide 170 mg I/ml in a man weighing 70 kg. When expressed in terms of mg iodine per ml CSF, these doses are in both man and rabbit equivalent to about 18 mg I/ml.

The absence of convulsions in all 20 rabbits injected with this above-stated dose accords with the known low seizure frequency seen in the clinical use of metrizamide, ranging between 0.008 (AHLGREN 1980) and 1.8% (SACKET et coll. 1977). In addition, the depressive changes in behaviour of 12 of these 20 rabbits accord with the clinical frequency of non-convulsive reactions - headache, nausea, vomiting - which generally range between 30 and 70% (AHLGREN 1975, 1980, SKALPE 1977, AMUNDSEN 1977, NICKEL & SALEM 1977, MARAVILLA et coll. 1979, PETTERSSON et coll. 1982). This might suggest that evaluation of changes in animal behaviour after a clinical dose of metrizamide provides some information about the frequency of adverse reactions in man after a clinical dose. It should be borne in mind that scoring changes in animal behaviour is, in

fact, scoring clinical symptoms in non-anaesthetized animals. The EEG method used in the present investigation did not provide statistically significant evidence of the observed non-convulsive reactions after injection of the "clinical dose" of metrizamide, but the EEG was abnormal (pattern 1) in 5 of 20 animals at the "clinical dose" (Table 1).

In a previous investigation (GOLMAN et coll. 1980) also using the observation of animal behaviour, contrast medium doses far above clinical level had to be employed to discriminate between the neurotoxic effects of iohexol and iopamidol. Thus, it can be concluded that EEG, as used in the present investigation, would not have been able to discriminate between contrast media less toxic than metrizamide at a clinical dose level. An improved EEG method including computerized quantitative frequency analysis of the EEG (SOVAK et coll. 1980, GONSETTE 1982, GIOUX et coll. 1982) might be able to make such a discrimination.

The possibility of an interaction between metrizamide and the alphaxolon/alphadolon anaesthesia is indicated from the results of the present study. This weak interaction has enabled the detection of signs of behavioural depression in previously anaesthetized animals that received a "clinical dose" of metrizamide (group 3). It might be possible to simultaneously use contrast media and drugs known for their ability to potentiate contrast medium neurotoxicity - for instance, phenothiazine derivatives (HINDMARSH et coll. 1975, GONSETTE & BRUCHER 1977) - so that signs of neurotoxicity appear at a lower contrast medium dose or at a higher frequency in animal experiments. A method utilizing such an interaction might make it possible to differentiate between the neurotoxic properties of contrast media less neurotoxic than metrizamide.

The increase of the metrizamide dose from 0.1 to 0.5 ml/kg in the present investigation caused a significant increase in

neurotoxic signs, which was revealed by both methods of detection. Such a dose response has previously been reported for both metrizamide (OFTEDAL 1973, GONSETTE & BRUCHER 1977) and ionic contrast media (MELARTIN 1970, SALVESEN 1981). Prediction of the adverse reactions in the clinical use of a contrast medium from its effects in animal experiments requiring high doses may be of low value. The reason is that it will be difficult to differentiate between neurotoxic effects caused by the contrast medium molecules and effects caused by the large contrast medium volume - with increase in the total osmolality in the subarachnoid space, dilution of electrolytes and other components of the CSF, and changes in the buffering capacity of the CSF.

The only method reported to be able to discriminate between the neurotoxic effects of metrizamide and the new generation of non-ionic contrast media at a clinical dose level is aversion conditioning in rats (SOVAK et coll. 1982). If other methods using various animal species do not become available that at a clinical dose can discriminate between the neurotoxic properties of contrast media less toxic than metrizamide, it will be highly urgent, then, to obtain knowledge about neurotoxic effects from altered CSF osmolality and effects associated with the dilution of CSF causing changes of CSF electrolyte content and buffering capacity.

Conclusion

There is a need for animal experimental methods that - ideally at a clinical dose level - can discriminate between the neurotoxic properties of subarachnoid contrast media less toxic than metrizamide and that enable prediction of adverse reactions that may be expected when using these contrast media clinically. EEG and evaluation of rabbit behaviour, as used in the present investigation, require high doses of contrast

medium above the clinical level.

If improved modifications of these methods - EEG with computerized frequency analysis or combined use of contrast media and drugs potentiating their neurotoxic effects - fail in differentiating between the least toxic contrast media at a clinical dose, it becomes increasingly urgent to investigate the interfering neurotoxic effects from large fluid volumes in the CSF, such as changes in CSF osmolality, electrolyte content, and buffering capacity.

REFERENCES

AHLGREN P.: Amipaque myelography. The side effects compared with Dimer X. *Neuroradiology* 9 (1975), 197.

- Early and late side effects of water-soluble contrast media for myelography and cisternography: A short review. *Invest. Radiol. (Suppl)* 15 (1980), 264.

AMUNDSEN P.: Metrizamide in cervical myelography. Survey and present state. *Acta Radiol. (Suppl) /Stockh/* 355 (1977), 85.

GIOUX M., ARNE P., PATY J. and CAILLE J.M.: Experimental model of neurotoxicity study of water-soluble contrast media. In: Amiel M. ed. *Contrast media in radiology*. p. 134. Springer-Verlag, Berlin Heidelberg New York 1982.

GOLMAN K., OLIVECRONA H., GUSTAFSON C., SALVESEN S., ALMÉN T. and MALY P.: Excitation and depression of non-anaesthetized rabbits following injection of contrast media into the subarachnoid space. *Acta Radiol. (Suppl) /Stockh/* 362 (1980), 83.

GONSETTE R.E.: Immediate and delayed neurotoxicity of newly synthesized contrast media demonstrated by EEG sequential analysis. In: Amiel M. ed. *Contrast media in radiology*. p. 141. Springer-Verlag, Berlin Heidelberg New York 1982.

- and BRUCHER J.M.: Potentiation of Amipaque. Epileptogenic activity by neuroleptics. *Neuroradiology* 14 (1977), 27.

- - Neurotoxicity of novel water-soluble contrast media for intrathecal application. *Invest. Radiol. (Suppl)* 15 (1980), 254.

- GYERMEK L. and SOYKA L.F.: Steroid anaesthetics. *Anaesthesiology* 42 (1975), 331.
- HILAL S.K., DAUTH G.W., HESS K.H. and GILMAN S.: Development and evaluation of a new water-soluble iodinated myelographic contrast medium with markedly reduced convulsive effects. *Radiology* 126 (1978), 417.
- HINDMARSH T., GREPE A. and WIDÉN L.: Metrizamide-phenothiazine interaction. Report of a case with seizures following myelography. *Acta Radiol. (Diagn) /Stockh/* 16 (1975), 129.
- HOLTZ E. AULIE MICHELET A. and JACOBSEN T.: Absorption after subarachnoid and subdural administration of iohexol, ^{51}Cr -EDTA and ^{125}I -albumin to rabbits. Submitted for publication to *AJNR*.
- MARAVILLA K.R., NEUWELT E.A. and DIEHL J.T.: Application of metrizamide in the radiographic evaluation of the neurologically diseased patient. *Neurosurgery* 5 (1979), 389.
- MELARTIN E.: Intracisternal toxicity of angio-graphic contrast media. Thesis, Turku (1970).
- NICKEL A.R. and SALEM J.J.: Clinical experience in North America with metrizamide. Evaluation of 1 850 subarachnoid examinations. *Acta Radiol. (Suppl) /Stockh/* 355 (1977), 409.
- OFTEDAL S.I.: Intraventricular application of water-soluble contrast media in cats. *Acta Radiol. (Suppl) /Stockh/* 335 (1973), 125.
- PETTERSSON H., FITZ C.R., HARWOOD-NASH D.C.F., ARMSTRONG E. and CHUANG S.H.: Adverse reactions to myelography with metrizamide in infants, children and adolescents. I: General and CNS effects. *Acta Radiol. (Diagn) /Stockh/* (in press).

- SACKETT J.F., STROTHER Ch.M., QUAGLIERI Ch.E., JAVID M.J.,
LEVIN A.B. and DUFF T.A.: Metrizamide-CSF contrast medium.
Radiology 123 (1977), 779.
- SALVESEN S.: Intracisternal toxicity in rats. A comparison of
the effects of iohexol, iopamidol, ioxaglate, and physiologi-
cal saline. Internal report by Nyegaard & Co., Oslo, Norway
1981.
- SIEFERT H.M., PRESS W.R. and SPECK U.: Tolerance to iohexol
after intracisternal, intracerebral and intraarterial
injection in the rat. Acta Radiol. (Suppl) /Stockh/ 362
(1980), 77.
- SIEGEL S.: Non-parametric statistics for the behavioral
sciences. McGraw-Hill, San Francisco 1956.
- SKALPE I.O.: Adverse effects of water-soluble contrast media
in myelography, cisternography and ventriculography. A
review with special reference to metrizamide. Acta Radiol.
(Suppl) /Stockh/ 355 (1977), 359.
- SOVAK M., RANGANATHAN R. and JOHNSON M.: Spectral analysis of
lapine EEG: neurotoxicologic evaluation of new nonionic
contrast media. Invest. Radiol. 15 (1980), 452.
- DEUTSCH J.A. and RANGANATHAN R.: Evaluation of intracranial
contrast media by aversion conditioning in rats. Invest.
Radiol. 17 (1982), 101.
- SPENCER C.P., CHRISMAN C.L., MAYHEW I.G. and KAUDE J.V.: Acute
neurotoxicity of iopamidol following subarachnoid applica-
tion. Proceedings of the Association of University Radiolo-
gists, annual meeting. Invest. Radiol. 15 (1980), 411.

FROM THE ¹DEPARTMENTS OF DIAGNOSTIC RADIOLOGY AND EXPERIMENTAL RESEARCH, MALMÖ GENERAL HOSPITAL, S-214 01 MALMÖ, SWEDEN, AND THE ²RESEARCH DEPARTMENT, NYEGAARD & CO. A/S, OSLO 4, NORWAY

INTERACTION BETWEEN CHLORPROMAZINE AND INTRATHECALLY
INJECTED NON-IONIC CONTRAST MEDIA IN NON-ANAESTHETIZED
RABBITS

P. Maly¹, H. Olivecrona¹, T. Almén¹ and K. Golman²

Supported in part by the Swedish Medical Research Council
(Project No. 3483)

Summary. During a 14-day period, 45 of 78 rabbits were treated intravenously with chlorpromazine ($5 \text{ mg kg}^{-1} \text{ day}^{-1}$). Metrizamide or iohexol was injected subarachnoidally and the neurotoxic effects of each substance on rabbit behaviour were evaluated for 3 hours in both chlorpromazine-treated and non-treated rabbits. Chlorpromazine increased both the excitative and the depressive effects of metrizamide and the depressive effects of iohexol. No signs of excitation were observed in chlorpromazine-treated and non-treated rabbits after subarachnoid injections of iohexol.

In spite of a free flow of clear fluid from the needle both before and after the intended subarachnoid injection, the radiography disclosed that 26 of 67 suboccipital injections inadvertently resulted in a subdural deposition of the contrast medium. Contrast medium effects following subdural injection significantly differed from those following subarachnoid injection.

Introduction

Generalized seizures after subarachnoid injection of the first non-ionic contrast medium metrizamide were first observed in animals under neuroleptanaesthesia (1, 2) and in a patient (3) who had been taking a neuroleptic. Therefore, medication of patients with neuroleptics is often discontinued before metrizamide myelography, although it has been claimed (4) that neuroleptics may even decrease the frequency of adverse reactions associated with metrizamide myelography.

A new non-ionic contrast medium - iohexol (Omnipaque^R) - is presently being clinically tested for myelography. In the subarachnoid space of animals, iohexol has produced less severe signs of acute neurotoxicity than the non-ionic media metrizamide (5, 6, 7) and iopamidol (5, 8).

It was our aim to investigate, in non-anaesthetized rabbits, whether or not the neuroleptic chlorpromazine interacts with subarachnoidally injected non-ionic contrast media - metrizamide and iohexol - and influences their neurotoxic effects.

Material and Methods

Seventy-eight albino rabbits (Swedish Land) of both sexes (median weight 1.5 kg, range 1.1 - 1.9 kg) were used. Daily, during a 14-day period, 45 rabbits were administered chlorpromazine (Klorpromex^R, Dumex) ($5 \text{ mg kg body-weight}^{-1}$) intravenously. The last dose of chlorpromazine was administered half an hour before the intrathecal injection of the contrast medium. Eleven of the chlorpromazine-treated rabbits served as a control group and their subarachnoid space was not punctured. The remaining 34 chlorpromazine-treated rabbits and the 33 untreated rabbits were randomly injected with metrizamide and iohexol at a dose of 1.0 ml kg^{-1} (both 370 mg l ml^{-1}).

Suboccipital puncture of the cisterna magna was performed with a 25-gauge needle (Butterfly^R, ABBOT A.S.). After obtaining a free flow of clear cerebrospinal fluid (CSF), the contrast medium was injected by an infusion pump at a rate of 0.4 ml min^{-1} . After the injection the rabbit was held vertically with its head down for 1 min to assure intracranial flow of the hyperbaric contrast medium. Within 3 min after the injection, a lateral radiograph of the skull and spine was made to determine the distribution of contrast medium within the subarachnoid space. Afterwards, the rabbits were placed in a 2 x 2 m cage for observation, which lasted 3 hours.

Evaluation of changes in animal behaviour was made by an observer who did not have knowledge of the injection of chlorpromazine and/or contrast media. Signs of excitation and

depression in animal behaviour were ranked according to a previously used system (5):

Excitation

Score

- | | |
|---|---|
| 0 | No effect |
| 1 | Hyperexcitability: a slight touching of the animal or a sudden noise evoked a muscular response of abnormal intensity |
| 2 | Spontaneous jerks with the head or local muscular twitching |
| 3 | One generalized seizure or myoclonic jerks of the whole body |
| 4 | More than one generalized seizure, recovery within 3 h |
| 5 | Many seizures without recovery within 3 h |

Depression

Score

- | | |
|---|---|
| 0 | No effect. A rabbit spontaneously moving around with normal motility and normal interest in other rabbits |
| 1 | Difficulties in moving around; recovery within 2 h |
| 2 | The animal lying pronely or on its side and unable to sit within 2 h |
| 3 | The animal lying pronely or on its side and unable to sit within 3 h |

The evaluation of the radiographs was carried out independently of the scoring. The statistical evaluation was performed with the two-sided Wilcoxon rank sum test ($P < 0.05$ was considered to be a level of significance).

Results

Chlorpromazine alone had a weak depressive effect on behaviour (score 1) of two rabbits in the control group. The behaviour of the remaining nine rabbits in this group was normal (Table 1).

In spite of a free flow of clear fluid from the needle before and after the suboccipital injection, the radiographs disclosed that 26 of 67 rabbits (39%) had inadvertently been injected into the subdural instead of the subarachnoid space (Table 1) (Fig. 1). In animals treated with chlorpromazine, the frequency of inadvertent subdural injections was as high as 53% (18 of 34 injections).

Subarachnoid injection

Chlorpromazine significantly increased the severity of excitative effects of metrizamide (Fig. 2). Among rabbits injected with metrizamide, all seven chlorpromazine-treated rabbits had repeated generalized seizures without recovery within 3 h (score 5), whereas the 12 non-treated rabbits had on the average only one seizure (median score 3) (Table 1). Chlorpromazine also increased the depressive effects of metrizamide, but the difference between non-treated and chlorpromazine-treated rabbits was not statistically significant (Fig. 2).

Chlorpromazine did not increase any excitative effects of iohexol (Fig. 1). Neither did iohexol cause any excitative effects in chlorpromazine-treated or in non-treated rabbits (Table 1). Chlorpromazine significantly increased the severity of the depressive effects caused by iohexol (Fig. 2); the depressive effects increased from a median score of 0 in non-treated rabbits to a median score of 3 in chlorpromazine-treated rabbits (Table 1).

Iohexol caused significantly less severe excitative effects than metrizamide in both non-treated and chlorpromazine-treated rabbits (Fig. 2). In non-treated rabbits, iohexol also caused significantly less severe depressive effects than metrizamide, whereas in chlorpromazine-treated animals, no significant difference in the depression scores was found between the iohexol group and the metrizamide group (Fig 2). However, the rabbits that received the maximum depression score (score 3) after injection of iohexol were lying pronely, whereas rabbits with the same score after injection of metrizamide were lying on their side.

Subdural injection

No seizures or other signs of excitation were observed in the 12 rabbits injected subdurally with metrizamide, but generalized seizures occurred in 14 of 19 rabbits (74%) injected with metrizamide subarachnoidally (Table 1).

In chlorpromazine-treated rabbits, subdural injection of metrizamide caused significantly less severe excitative effects (score 0 in all 8 rabbits) than subarachnoid injection (score 5 in all 7 rabbits) (Fig. 3). Further, subdural injection of metrizamide produced slightly less severe depressive effects (median score 2.5) than subarachnoid injection (median score 3), but the difference was not statistically significant (Fig. 3). However, the depressive effects of subdural injection of metrizamide were most severe immediately after the injection, whereas the depressive effects after subarachnoid injection occurred later.

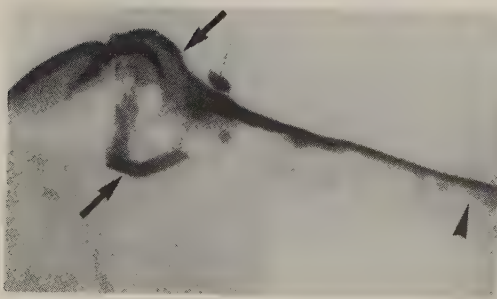
Neither subdural nor subarachnoid injection of iohexol caused any excitative effects in chlorpromazine-treated rabbits. In these animals, subdural injection of iohexol caused significantly less severe depressive effects (median score 0 in 10 rabbits) than subarachnoid injection (median score 3 in 9 rabbits) (Fig. 3).



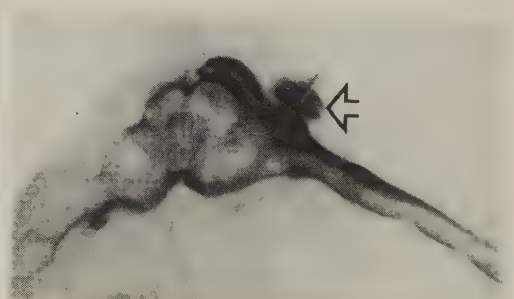
a



b



c



d

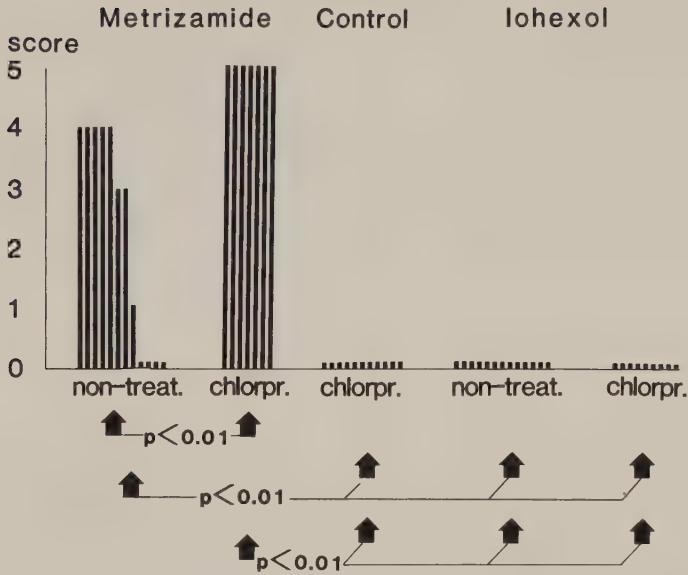
Fig. 1. Subtraction radiographs demonstrating three types of possible deposition of contrast media following a percutaneous puncture of the cisterna cerebellomedullaris (cisterna magna) in rabbit.

a/ Subarachnoid injection. Continuous delineation of spinal cord. Spinal vessels often distinguishable (long thin arrow). Cisterna magna continuing rostrally into the fourth ventricle (bent open arrow). Delineation of cerebellum, cerebral hemispheres, bulbus olfactorius and of basal cisterns.

b-c/ Subdural injection: the contrast medium is entirely confined to the subdural space. Asymmetric distribution of contrast medium in the spinal canal. Lack of definition of spinal cord. Absence of contrast medium within the ventricles and cranial subarachnoid space. Pathognomonic changes: Smooth scalloping of the anterior aspect of the contrast medium column (arrow-head); "punched-out" defects caused by connection between arachnoid membrane and the inner layer of dura mater (thin arrow); sharply delineated subdural deposition of the contrast medium in the intracranial occipital and basal posterior regions (arrow).

d/ Combined deposition - subarachnoid and subdural - following intentional successive withdrawal of the needle. The last portion of contrast medium injected extraspinally in the soft tissue of the neck (open arrow). In spite of this, there was a free flow of a clear fluid (probably only contrast medium) from the needle at the end of this injection. Thus, a free flow of fluid does not mean correct needle position.

EXCITATION



DEPRESSION

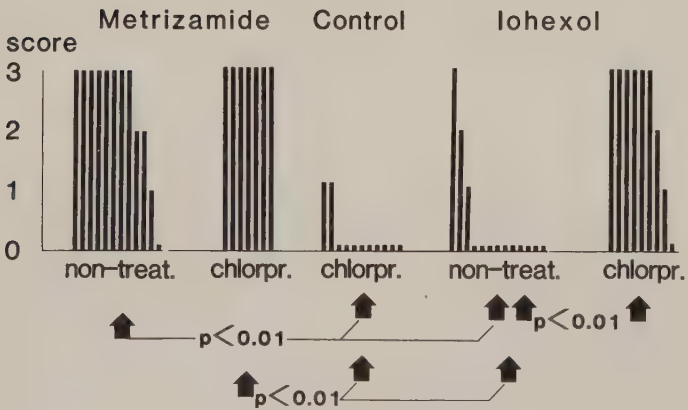
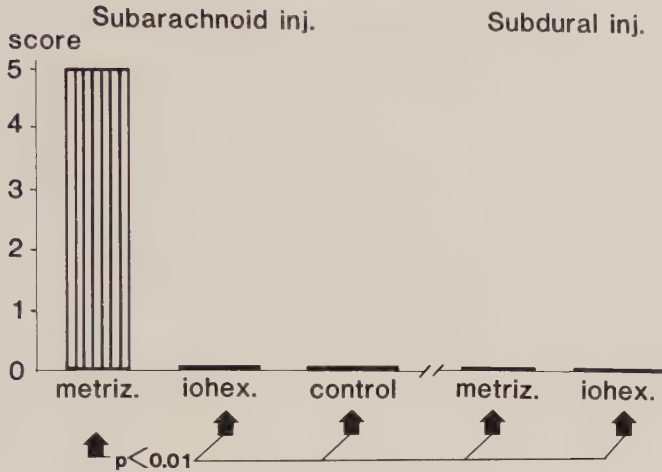


Fig. 2. Subarachnoid injection. Statistical evaluation of scores of excitation and depression caused by subarachnoidally injected metrizamide and iohexol in non-anaesthetized rabbits not treated or treated with chlorpromazine (chlorpr.) for 14 days. Each column depicts one rabbit.

EXCITATION



DEPRESSION

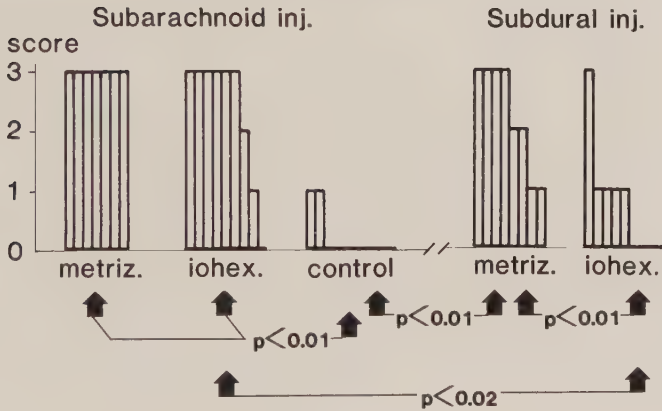


Fig. 3. Subarachnoid versus subdural injection. Statistical evaluation of scores of excitation and depression caused by metrizamide and iothexol injected into the subarachnoid and the subdural the space of non-anaesthetized rabbits treated intravenously with chlorpromazine. Each column depicts one rabbit.

Discussion

Iohexol injected subarachnoidally at a dose about 10 times higher than the dose currently used in clinical myelography did not cause signs of excitation in the behaviour of any rabbit despite the fact that 41% of the rabbits were treated with chlorpromazine. Such chlorpromazine treatment significantly increased the excitative effects of metrizamide to the highest level (score 5) that can be demonstrated by our animal model (Fig. 2). The absence of excitative effects of iohexol in chlorpromazine-treated rabbits confirms and reinforces the impression gained from earlier animal experiments (5, 6) that subarachnoidally injected iohexol causes less severe excitative effects than metrizamide. The weak depressive effects of subarachnoidally injected iohexol - significantly lower than those of metrizamide - were, however, increased by the effect of chlorpromazine.

The extent to which the present investigation can predict the occurrence of clinical side-effects following iohexol myelography depends on a knowledge of the mechanisms involved in evoking such side-effects. Changes in animal behaviour may be induced both by the iohexol molecules alone and by the hyperosmolality of the iohexol solution. The relative quantitative roles of molecular toxicity and hyperosmolality are not known, but they are particularly important when large volumes of iohexol are injected in the subarachnoid space.

It has been suggested (9) that the net neurotoxic effect of a contrast medium solution is the sum of two opposing effects: an excitative effect due to the molecular toxicity and a depressing effect due to the hyperosmolality. The osmolality of an iohexol solution at 370 mg I ml^{-1} is 3.2 times higher than that of rabbit cerebrospinal fluid. It could thus be argued that iohexol molecules could possibly cause some weak excitative effects, but in the present investigation these were

obscured by depressive effects of the high osmolality of the solution. For this reason, excitative effects of an iohexol solution in the subarachnoid space have been investigated after injection of the same number of iohexol molecules: both as a solution iso-osmolar to CSF and as a solution hyperosmolar to CSF (7). In that study (7), neither an iso-osmolar nor a hyperosmolar solution produced any signs of excitation. Results from other animals experiments (5, 6, 7) and from the present investigation may be used to predict the following clinical advantages of using iohexol instead of metrizamide in the subarachnoid space:

- 1) lower risk of generalized seizures in patients treated with neuroleptics;
- 2) lower frequency and severity of adverse reactions corresponding to "signs of excitation" in animal behaviour, such as generalized or focal seizures, muscle cramps, fasciculations or myoclonia;
- 3) lower frequency and severity of other adverse reactions that possibly may correspond to "signs of depression" in behaviour, such as headache and backache, malaise, weakness, dizziness, etc.

The effects of the two intrathecally injected contrast media were significantly different (Fig. 3) if they had intentionally been injected into the subarachnoid space or inadvertently injected into the subdural space. In animal research, no attention has been given to inadvertent subdural injection as a possible source of error giving spurious results. In man, the frequency of inadvertent extra-arachnoid injections may be as high as 10-11% (10, 11). After a subdural injection, the substance is deposited between two thecae, the dura mater and arachnoid membrane (Fig. 1). Of these thecae, only the arachnoid membrane has histologic characteristics compatible with a barrier function (12, 13).

In the present investigation, it was the total absence of

excitative effects after subdural injection of metrizamide in chlorpromazine-treated rabbits (Fig. 3) that most strikingly has shown that no or only a small number of metrizamide molecules reached the sensitive structures of the central nervous system. This observation may be regarded as additional functional evidence supporting the opinion (12, 14) that it is the arachnoid membrane, and not the inner surface of the dura mater, that constitutes the blood-CSF border of the blood-brain barrier. That the arachnoid membrane acts as a barrier against contrast medium molecules is also indicated by findings showing that iohexol is eliminated significantly more rapidly from the subdural space of the rabbit than from the subarachnoid space (15).

Appearance of a "fast" and a "slow" type of resorption has been noted also in man after repeated lumbar "intrathecal" injections of methotrexate (16). The possibility of an inadvertent subdural injection that may lead to the absence of an intended therapeutic effect and that might be the cause of the rapid resorption of methotrexate has not been considered, although several data in that study (16) may support this possibility. A free flow of CSF both before and after the "intrathecal" injection of any substance is not proof of a correct subarachnoid deposition, because the flow of CSF would also be equally free both before and after a subdural injection. To be absolutely sure of a subarachnoid deposition of any substance, the contrast medium must be concomitantly injected and checked with radiography. In studies not using contrast media, the possibility of an inadvertent subdural injection must be borne in mind when unexpected or controversial results - e.g. bimodal distribution of measured data - are obtained following intrathecal injection.

In the present investigation, the combined action of chlorpromazine and metrizamide produced signs of excitation in rabbit behaviour corresponding to a median score of 5, which

was higher than the sum of median scores ($3 + 0 = 3$) produced by each compound separately (Fig. 2, Table 1). Thus, chlorpromazine, with its potentiating effect, not only increased the excitative effects of metrizamide but also enhanced the depressive effects of iohexol: the median score was higher when iohexol and chlorpromazine were given simultaneously (score 3) than the sum of median scores of effects produced by these compounds given separately ($0 + 0 = 0$) (Fig. 2, Table 1). By simultaneous administration of phenothiazine derivatives and of contrast media, the ability of experimental methods to discriminate between the effects of contrast media can be increased.

However, the interaction between chlorpromazine and the contrast medium can also decrease differences in effects between different contrast media, which is demonstrated in the present investigation: when injected subarachnoidally into rabbits not treated with chlorpromazine, metrizamide produced severe depressive effects, with a median score of 3, whereas iohexol produced significantly less severe depressive effects (median score 0). This difference in depressive effects between the two media decreased and became non-significant in rabbits treated with chlorpromazine in which both media produced the same median depression score: 3 (Fig. 2, Table 1).

Conclusions

The risk of seizures and possibly of other untoward side-effects that are associated with clinical investigations of the subarachnoid space might be decreased by using iohexol (Omnipaque^R) instead of metrizamide (Amipaque^R).

Whenever a substance is injected intrathecally, attention should be directed to the possibility of obtaining spurious results owing to an inadvertent subdural injection instead of

the intended subarachnoid injection.

In experimental research, medication of animals with drugs that potentiate neurotoxic effects of contrast media may be tried to increase the ability of the experimental model to differentiate between compounds that may otherwise exhibit similar neurotoxic effects.

REFERENCES

1. Grepe A, Widén L (1973) Neurotoxic effect of intracranial subarachnoid application of metrizamide and meglumine iocarmate. An experimental investigation in dogs in neurolept analgesia. Acta Radiol /Suppl/ (Stockh) 335:-102-118
2. Grepe A, Widén L (1973) Effects of cisternal application of metrizamide. An experimental investigation in dogs in N₂O analgesia with and without Halothane. Acta Radiol /Suppl/ (Stockh) 335:119-124
3. Hindmarsh T, Grepe A, Widén L (1975) Metrizamide-phenthiazine interaction. Report of a case with seizures following myelography. Acta Radiol /Diagn/ (Stockh) 16:129-134
4. Ahlgren P (1980) Early and late side effects of water-soluble contrast media for myelography and cisternography: A short review. Invest Radiol /Suppl/ 15:264-266
5. Golman K, Olivecrona H, Gustafson C, Salvesen S, Almén T, Maly P (1980) Excitation and depression of non-anaesthetized rabbits following injection of contrast media into the subarachnoid space. Acta Radiol /Suppl/ (Stockh) 362:83-86
6. Siefert HM, Press WR, Speck U (1980) Tolerance to iohexol after intracisternal, intracerebral and intraarterial injection in the rat. Acta Radiol /Suppl/ (Stockh) 362:77-81
7. Maly P, Almén T, Golman K, Olivecrona H (1983) Excitative effects of subarachnoidally injected iso- and hyperosmolar iohexol and metrizamide in anaesthetized rabbits. To be published

8. Gonsette RE (1982) Immediate and delayed neurotoxicity of newly synthesized contrast media demonstrated by EEG sequential analysis. In: Amiel M, ed. Contrast media in radiology. Springer-Verlag, Berlin Heidelberg New York. p. 141-148

9. Hilal SK, Dauth GW, Hess KH, Gilman S. (1978) Development and evaluation of a new water-soluble iodinated myelographic contrast medium with markedly reduced convulsive effects. Radiology 126:417-422

10. Jones MD, Newton TH (1963) Inadvertent extra-arachnoid injections in myelography. Radiology 80:818-822

11. Larson GM, Schall GL, DiChiro G (1972) The unsuccessful injection in cisternography: Incidence, cause and appearance. In: Harbert JC, ed. Cisternography and Hydrocephalus. A symposium. Charles C Thomas, Springfield, Illinois. p. 153-171

12. Waggener JD, Beggs J (1967) The membranous covering of neural tissues: An electron microscopy study. J Neuropathol Exp Neurol 26:412-426

13. Fishman RF (1980) Cerebrospinal fluid in diseases of the nervous system. W.B. Saunders Co, Philadelphia, London, Toronto. p. 13-18

14. Oldendorf WH (1972) Cerebrospinal fluid formation and circulation. Progr Nucl Med 1:336-358

15. Holtz E, Aulie Michelet A, Jacobsen T. Absorption after subarachnoid and subdural administration of iohexol, ⁵¹Cr-EDTA and ¹²⁵I-albumin to rabbits. Submitted for publication in AJNR

16. Lankelma J, Lippens RJJ, Drenthe-Schonk A, Termond EFS, van der Kleijn E. (1980) Change in transfer rate of methotrexate from spinal fluid to plasma during intrathecal therapy in children and adults. Clin Pharmacokinet 5:465-475

FROM THE ¹DEPARTMENTS OF DIAGNOSTIC RADIOLOGY AND EXPERIMENTAL
RESEARCH, MALMÖ GENERAL HOSPITAL, S-214 01 MALMÖ, SWEDEN, AND
THE ²RESEARCH DEPARTMENT, NYEGAARD & CO. A/S, OSLO 4, NORWAY

EXCITATIVE EFFECTS IN ANAESTHETIZED RABBITS FROM
SUBARACHNOIDALLY INJECTED ISO- AND HYPEROSMOLAR
SOLUTIONS OF IOHEXOL AND METRIZAMIDE

P. Maly¹, T. Almén¹, K. Golman² and H. Olivecrona¹

Supported in part by the Swedish Medical Research Council
(Project no. 3483)

Summary. Equimolar doses of iohexol and metrizamide (0.48 mmol per kg body-weight = 185 mg I/kg), administered in solutions iso- and hyperosmolar to the CSF, were injected subarachnoidally into rabbits under pentobarbital anaesthesia. No excitative effects of iohexol were observed in the behaviour of the rabbits during a 3-hour period immediately after the injection. Metrizamide caused significantly more severe excitative effects, ranging from hyperexcitability to repeated generalized seizures. The frequency and severity of excitative effects were independent of the osmolality of the metrizamide solutions injected.

Introduction

Iohexol (Omnipaque^R) - a monomeric non-ionic contrast medium - is presently being clinically tested for myelography. After injections into the subarachnoid space of animals, iohexol has been shown to cause significantly fewer and less severe adverse reactions than metrizamide, iopamidol, and ioglundide (P297) (1-4). After subarachnoid administration of iohexol, no paroxysmal EEG activity, seizures, or excitative effects on animal behaviour have been reported.

Large doses of hyperosmolar contrast media had to be used in these studies to produce the adverse reactions at a higher frequency than they occur clinically. Hyperosmolality alone may, however, decrease the irritative effects of contrast medium molecules on electrophysiologic events in the central nervous system (CNS) (5, 6). Thus, it is important to exclude that some superimposed depressive effect of hyperosmolality could have been responsible for the total absence of excitative effects of iohexol in the above-mentioned animal experiments.

The aim of the present study was to investigate whether

hyperosmolality or a large volume of contrast medium solution injected into the subarachnoid space increases or decreases the excitative effects that are produced by contrast medium molecules. Excitative effects were evaluated for 3 h in rabbits injected subarachnoidally with iso- and hyperosmolar solutions of iohexol and metrizamide. It was desirable to preserve a hyperosmolar contrast medium bolus within the intracranial subarachnoid space for as long a period as possible. Therefore the animals were anaesthetized in order to preclude animal movements that might accelerate mixing and dilution of the contrast medium bolus with the cerebrospinal fluid (CSF).

Material and Methods

Fifty-two albino rabbits (Swedish Land) of both sexes weighing 1.1 - 2.2 kg (median 1.3 kg) were used for the investigation. Rabbits with blood-stained CSF after the puncture or those with a subdural deposition of contrast medium were excluded and replaced (totally 18 rabbits).

The animals were during the entire experiment, which lasted for 3 h, under light intravenous pentobarbital anaesthesia (Mebumal^R) with the corneal reflex preserved. No puncture of the subarachnoid space was performed on the 7 rabbits in the control group. The remaining 45 rabbits were, after anaesthetization, fixed with a belt around their pelvis in the right decubitus position on a plexiglass table with the head end lowered 40° to ensure successive replacement of the CSF within the intracranial subarachnoid space by the hyperbaric contrast media solutions. A suboccipital puncture of the cisterna magna was performed with a 25-gauge needle (Butterfly^R, ABBOT A.B.); and after achieving a free flow of clear CSF from the needle, contrast medium was injected with an infusion pump at a rate of 0.4 ml/min. Both contrast media - iohexol and metrizamide -

were injected in solutions iso-osmolar to CSF (144 and 166 mg I/ml, respectively) and in hyperosmolar solutions (370 mg I/ml) at volumes adjusted, so that the rabbits received the same number of contrast medium molecules per kg of body-weight, i.e., 0.48 mmol contrast medium/kg.

The rabbits, except the 7 animals in the control group, were randomly injected with the following contrast media preparations: iso-osmolar iohexol (10 animals, 144 mg I/ml, 1.28 ml/kg), hyperosmolar iohexol (15 animals, 370 mg I/ml, 0.5 ml/kg), iso-osmolar metrizamide (10 animals, 166 mg I/ml, 1.11 ml/kg), hyperosmolar metrizamide (10 animals, 370 mg I/ml, 0.5 ml/kg), (Table 1).

During the contrast medium infusion, one series of films was exposed in submento-vertical projection over the head and in antero-posterior projection over the spine using an AOT film changer (Siemens-Elema A.B.) and an 0.1 mm focal spot to study the replacement of the CSF by contrast medium within the subarachnoid space. Following completion of the infusion, a lateral roentgenogram of the head and spine was made and studied immediately to exclude those animals in which the contrast media were deposited subdurally. The animals were then placed in the right decubitus position with the head at the same level as the spine, for behavioural observations.

Signs of excitation in animal behaviour were observed during a 3-hour period after the injection of the anaesthetic in the control group or of the contrast medium in the remaining anaesthetized animals. The observer did not have knowledge of the suboccipital puncture and/or of the contrast medium preparation injected. Limb jerks were noted and recorded with respect to side difference (right or left). The excitative effects were ranked according to a predetermined scoring system:

Excitation

Score

- | | |
|---|---|
| 0 | No effect |
| 1 | Hyperexcitability: a slight touching of the animal evoked a muscular response of abnormal intensity |
| 2 | Spontaneous jerks with the head or local muscular twitching |
| 3 | One generalized seizure or myoclonic jerks of the whole body |
| 4 | More than one generalized seizure, recovery within 3 h |
| 5 | Many seizures without recovery within 3 hours |

The statistical analysis of the obtained scores was performed with the two-sided Wilcoxon rank sum test. $P < 0.05$ was considered a level of significance.

Results

Radiography. In all animals, the hyperbaric contrast medium successively filled the declivous (right) side of the intracranial subarachnoid space, thereafter also the acclivous (left) side; and finally, when large volumes of contrast medium were injected (both iso-osmolar solutions), also the cervical and thoracic subarachnoid space became filled (Fig. 1).

Excitative effects. No excitative effects were detected in the control group of rabbits that had only been anaesthetized (Table 1).

Iohexol did not cause any signs of hyperexcitability when administered either as an iso-osmolar solution or as a hyper-osmolar solution. The median excitation score was 0.0 in both

groups (Table 1). Excitative effects ranging from hyperexcitability (score 1) to repeated generalized seizures (score 5) were exclusively produced by the two metrizamide preparations (Table 1). There was no significant difference between the excitative effects induced by the iso-osmolar and the hyperosmolar solution of metrizamide: median excitation score 1.5 v. 2.0 (Fig. 2).

Metrizamide, thus, caused significantly more frequent and severe excitative effects than iohexol irrespective of osmolality and/or volume of their solutions (Fig. 2).

Hyperosmolality alone of a small volume or a large volume alone of an iso-osmolar solution (Table 1) caused no excitative effects in groups injected with iohexol. Hyperosmolality alone of a small volume and a large volume alone of iso-osmolar solution of metrizamide produced the same net excitative effect (Fig. 2).

Tonic and/or clonic jerks were observed in all those rabbits injected with metrizamide, which received an excitation score of 2 and above. These jerks commenced at about 60 - 90 min after the metrizamide injection and occurred independently of the depth of anaesthesia. The jerks were exclusively (4 rabbits) or predominantly (6 rabbits) observed in the left limbs (Table 2), i.e., contralaterally to the presumed accumulation of the hyperbaric contrast medium on the declivous right side of the skull. Only one rabbit injected with hyperosmolar metrizamide displayed paw jerks solely on the ipsilateral right side (Table 2). One rabbit from the same group did not exhibit any jerks in the paws, but only with its head (Table 2).

Table 1. Scores of excitative effects caused by subarachnoid injection of equimolar doses of iohexol and metrizamide per kg body-weight in rabbits anaesthetized with pentobarbital

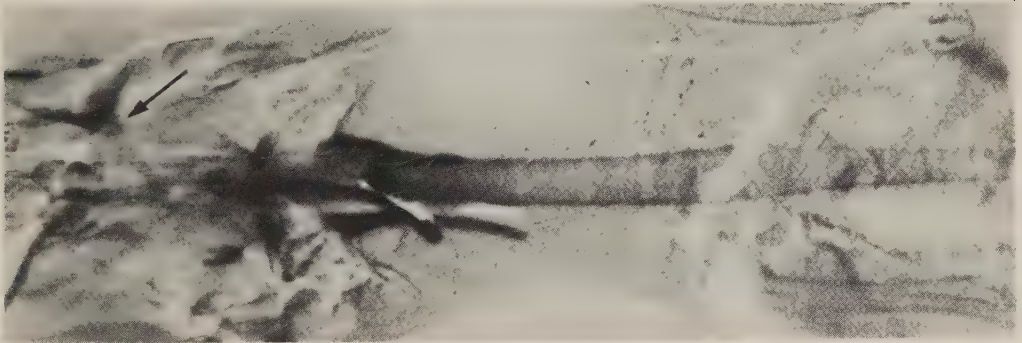
Contrast medium	Control	Iohexol	Iohexol	Metriz.	Metriz.
Concentration: (mg I/ml)	-	144	370	166	370
Osmolality: (mmol/kg H ₂ O)	-	300	980	300	580
Dose: (ml/kg animal)	-	1.28	0.50	1.11	0.50
Dose: (mmol/kg animal)	-	0.48	0.48	0.48	0.48
No. of animals	7	10	15	10	10
Excitation score			0		
			0		
			0		
			0		
		0	0	0	0
		0	0	0	0
		0	0	1	0
0	0	0	0	1	2
0	0	0	0	1	2
0	0	0	0	2	2
0	0	0	0	2	2
0	0	0	0	2	3
0	0	0	0	5	3
0	0	0	0	5	5



Fig. 1a. The course of subarachnoid injection of 0.6 ml of iothexol 370 mg I/ml. Predominant filling of the declivous parts of the subarachnoid space (thin arrows).

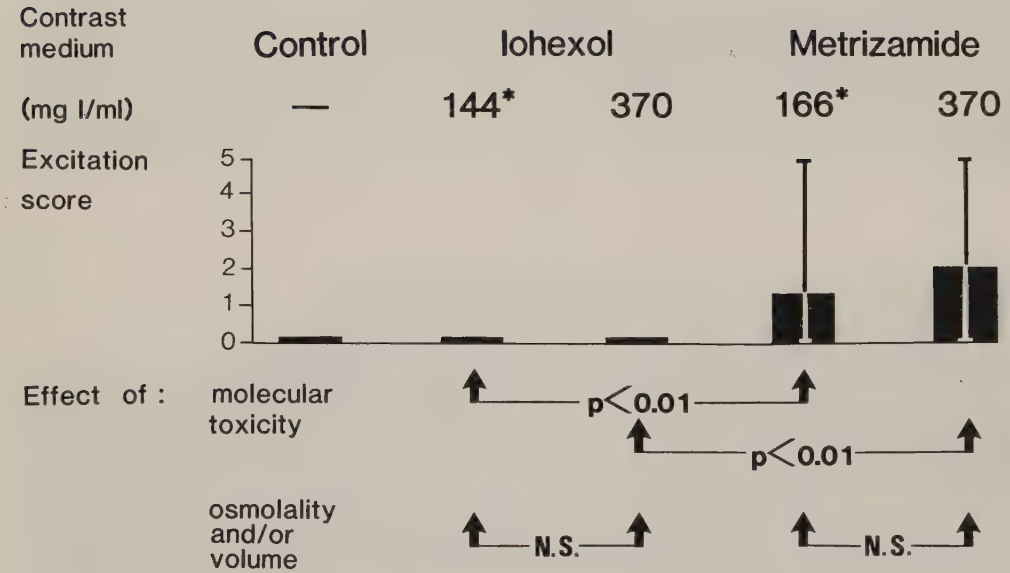


b



c

Fig. 1b and c. The end of injection of 0.6 ml of iohexol 370 mg I/ml (b) and of 1.22 ml of metrizamide 166 mg I/ml (c). The hyperbaric contrast media entered the intracranial subarachnoid space before the spinal one. Filling of the optical nerve sheath (arrow). (a, b, and c in submento-vertical projection)



* Solutions iso-osmolar to CSF

Fig. 2. Scores of excitative effects (median and range) in the behaviour of anaesthetized rabbits injected subarachnoidally with contrast media. P-value of differences in scores between groups.

Table 2. Tonic and/or clonic jerks of limbs in anaesthetized rabbits lying in right lateral position after subarachnoid injection of metrizamide. Acclivous limb = upper situated = left fore- or hind limb. Declivous limb = lower situated = right fore- or hind limb

Metrizamide mg I ml	Excitation score	Jerks of		
		acclivous limbs		declivous limbs
166	2	+	>	-
	2	+	>	-
	2	+	>	+
	5	+	>	+
	5	+	>	+
370	2*	-		-
	2	+	>	-
	2	+	>	-
	2	+	>	+
	3	-	<	+
	3	+	>	+
	5	+	>	+

Acclivous > declivous

10

Declivous > acclivous

1

* Jerks with the head only

Discussion

Molecular toxicity versus hyperosmolality. Two main characteristics of a water-soluble contrast medium - the chemotoxicity of its molecules and the osmolality of its solution - are generally considered to be responsible for most adverse reactions following injection of contrast medium into the subarachnoid space. The chemotoxicity of contrast medium molecules is believed to cause excitative effects on the electrophysiologic events in the CNS (5-7), whereas hyperosmolality alone has been shown to act depressingly on the EMG (8), the evoked corticospinal responses (5), the spinal root reflexes (6), and the evoked extracellular field potentials in rat hippocampus (9).

The direct action of molecular toxicity and hyperosmolality at the cellular level is poorly understood, but it must be emphasized that both types of mechanisms simultaneously participate in those changes of CNS function that are manifested in animal behaviour as "depression" and/or "excitation". The quantitative relations of the effects of these two mechanisms and the result of their mutual interaction are not known, but may be particularly important when large volumes of hyperosmolar contrast medium are used, such as in animal experiments.

All the new non-ionic monomeric contrast media - iopamidol, ioglunide (P297), iohexol, and ioglucomide - have higher osmolality than the first clinically used non-ionic medium metrizamide. In spite of their higher osmolality, these contrast media have fewer and weaker excitative effects than metrizamide when subarachnoidally injected in animals (1, 4, 5, 10-13). The maximum difference in excitative effects caused by the least osmolar medium - metrizamide - and the most hyperosmolar of those media - iohexol - has been obtained in non-anaesthetized rabbits treated with chlorpromazine. When injected subarachnoidally in these animals, hyperosmolar metriz-

amide (370 mg I/ml) caused repeated generalized seizures in all the animals, whereas hyperosmolar iohexol (370 mg I/ml) did not cause any signs of excitation in any animal (4).

It can be argued that the depressive effects of hyperosmolality alone could have obscured such effects of molecular toxicity of iohexol on the electrophysiologic events in the CNS that are displayed in animal behaviour as "excitation". However, no such effects of iohexol molecules were elicited by the present investigation, as iohexol did not cause any signs of excitation though injected in solution iso-osmolar to the CSF (Table 1). Moreover, the hyperosmolality of the solution neither decreased nor increased the excitative effects caused by the chemotoxicity of metrizamide molecules (Fig. 2). These results thus suggest that it is the molecular toxicity of the monomeric non-ionic contrast media, and not the hyperosmolality of their solutions, that is the major factor determining their excitative effects on the central nervous system after their subarachnoid administration.

The results of the present investigation reinforce impressions gained from earlier animal experiments (1-4) that very small, if any, clinical side-effects corresponding to the "excitative effects" in animal behaviour may be expected when iohexol is subsequently used in the subarachnoid space of man.

Paw jerks and/or local muscular twitching, which represent the second degree on our rank scale of excitative effects, have previously been observed after subarachnoid injection of non-ionic contrast media metrizamide, iopamidol, and ioglundide (P297) (1, 4, 14). In these experiments, conducted on non-anaesthetized rabbits, the contrast media have been distributed on both the right and the left side of the intracranial subarachnoid space and also within the spinal subarachnoid space. Until the present investigation, it has not been clear whether the jerks represent a certain degree of neurotoxic

effects of non-ionic contrast media on subcortical or cortical structures of the brain just below the degree causing generalized seizures or whether the jerks should be attributed to a local neurotoxic effect on the spinal cord and/or on the nerve roots.

Such locally provoked jerks have been observed, e.g., in rabbits (15) and in man (16) after spinal subarachnoid administration of ionic contrast media (iothalamate). When injected selectively into the lumbar subarachnoid space of the cat, the ionic contrast media have caused both tonic and clonic spasms in the tail and hind limbs, whereas the non-ionic metrizamide has neither caused muscle spasms nor electromyographic changes (17). Jerks of the left forepaw have, however, been recorded in two cats that had had "some sharp components" on their EEG (18) after injection of metrizamide into the right lateral ventricle.

In the present investigation, it can be assumed that the hyperbaric contrast medium mainly accumulated within the right declivous parts of the subarachnoid space while the newly formed CSF - with an hourly turnover of about 25 per cent - has stratified above the contrast medium bolus, thus, within the left acclivous parts of the subarachnoid space. With respect to such a side difference in contrast medium distribution, the jerks occurred on the side contralateral to the side of maximum concentration of contrast medium (Table 2).

From the observations made by SKALPE (17) and OFTEDAL (18) and from the present investigation, it could be concluded that jerks from limbs after subarachnoid administration of metrizamide, and possibly other non-ionic contrast media, are elicited from the brain. Thus, the jerks may represent a subcortical or cortical focal seizure activity induced by non-ionic contrast media rather than manifestations of some irritation of the spinal cord or nerve roots.

Conclusions

It is the chemotoxicity of the molecule and not the hyperosmolality of the solution that mainly determines signs of CNS excitation following subarachnoid administration of monomeric non-ionic contrast media.

Focal muscle cramps, fasciculations, and myoclonia, which very rarely occur after subarachnoid injections of metrizamide in man, may have their origin in focal convulsive activity in the brain rather than as a result of a direct irritation of the spinal cord or nerve roots.

References

1. Golman K, Olivecrona H, Gustafson C, Salvesen S, Almén T, Maly P (1980) Excitation and depression of non-anaesthetized rabbits following injection of contrast media into the subarachnoid space. *Acta Radiol /Suppl/ (Stockh)* 362: 83-86
2. Siefert HM, Press WR, Speck U (1980) Tolerance to iohexol after intracisternal, intracerebral and intraarterial injection in the rat. *Acta Radiol /Suppl/ (Stockh)* 362: 77-81
3. Gonsette RE (1982) Immediate and delayed neurotoxicity of newly synthesized contrast media demonstrated by EEG sequential analysis. In: Amiel M (ed) *Contrast media in radiology*. Springer-Verlag, Berlin, Heidelberg, New York, pp 141-148
4. Maly P, Olivecrona H, Almén T, Golman K (1983) Interaction between chlorpromazine and intrathecally injected non-ionic contrast media in non-anaesthetized rabbits. In manuscript.
5. Hilal SK, Dauth GW, Hess KH, Gilman S (1978) Development and evaluation of a new water-soluble iodinated myelographic contrast medium with markedly reduced convulsive effects. *Radiology* 126: 417-422
6. Bryan RN, Dauth GW, Gilman S, Hilal SK (1981) Effects of radiographic contrast agents on spinal cord physiology. *Invest Radiol* 16: 234-239
7. Hilal SK, Dauth GW, Burger LC, Gilman S (1977) Effect of isotonic contrast agents on spinal reflexes in the cat. *Radiology* 122: 149-155

8. Piwonka RW, Healey JF, Rosenberg FJ (1976) Intrathecal tolerance of metrizamide in chloralose anesthetized cats. *Invest Radiol* 11: 182-186
9. Hershkowitz N, Bryan RN (1982) Neurotoxic effects of water-soluble contrast agents on rat hippocampus: extra-cellular recordings. *Invest Radiol* 17: 271-275
10. Sovak M, Siefert HM, Ranganathan R (1980) Combined methods for assessment of neurotoxicity: Testing of new nonionic radiographic media. *Invest Radiol /Suppl/* 15: 248-253
11. Sovak M, Ranganathan R, Johnson M (1980) Spectral analysis of lapine EEG: neurotoxicologic evaluation of new nonionic contrast media. *Invest Radiol* 15: 452-456
12. Sovak A, Ranganathan R, Kerber C (1982) A nonionic, isotonic dimer iotrol: a new contrast medium for the intrathecal space. A review of pharmacological evaluation. In: Amiel M (ed) *Contrast media in radiology*. Springer-Verlag, Berlin Heidelberg, New York, pp 123-128
13. Hopkins RM, Adams MD, Lau DHM, Creighton JM, Brooke Hoey G (1981) Ioglucomide: a new nonionic myelographic agent. Preclinical studies. *Radiology* 140: 713-717
14. Maly P, Elmqvist D, Almén T, Golman K (1983) Comparison between EEG and observation of rabbit behaviour in evaluation of subarachnoid neurotoxicity of metrizamide. In manuscript.
15. Praestholm J (1972) Water-soluble contrast medium myelography in the experimental animal. A preliminary report. *Acta Radiol /Diagn/ (Stockh)* 13: 848-859

16. Gonsette R (1971) An experimental and clinical assessment of water-soluble contrast medium in neuroradiology. A new medium-Dimer-X. Clin Radiol 22: 44-56
17. Skälpe IO (1973) Myelography with metrizamide, meglumine, iothalamate and meglumine iocarmate. Acta Radiol /Suppl/ (Stockh) 355: 57-64
18. Oftedal SI (1973) Intraventricular application of water-soluble contrast media in cats. Acta Radiol /Suppl/ (Stockh) 335: 125-132

FROM THE DEPARTMENTS OF DIAGNOSTIC RADIOLOGY, EXPERIMENTAL
RESEARCH, AND CLINICAL CHEMISTRY, MALMÖ GENERAL HOSPITAL,
S-214 01 MALMÖ, SWEDEN

CSF CATION CHANGES FOLLOWING SUBARACHNOID INJECTION OF
IOHEXOL AND METRIZAMIDE IN RABBITS

P. Maly and G. Fex

Supported in part by the Swedish Medical Research Council
(Project No. 3483)

SUMMARY

Forty anaesthetized rabbits were injected subarachnoidally with equimolal doses, 0.48 mmol/kg body-weight (= 185 mg I/kg), of iohexol and metrizamide, administered in solutions iso- and hyperosmolal to the CSF. Concentrations of Na, K, Ca, and Mg in the CSF were determined before and 3 hours after the contrast medium injections. Iso-osmolal iohexol caused a uniform decrease in all four cations, which was believed to be a simple dilution. The hyperosmolal solution of iohexol caused a significant relative increase in Ca compared with Na, K, and Mg. Both metrizamide solutions caused an increase in Ca over its pre-injectional level and a significant relative increase of both Ca and Mg as compared with Na and K. The changes caused by the three latter solutions were consistent with an increase in the blood-brain barrier permeability.

Introduction

Among non-ionic monomeric contrast media developed after metrizamide, iohexol (Omnipaque^R) seems to have the lowest neurotoxicity after subarachnoid administration (GOLMAN et coll. 1980, GONSETTE 1982). To provoke detectable changes in animal behaviour, doses of iohexol must approach the volume of the cerebrospinal fluid (CSF) (GOLMAN et coll. 1980, MALY et coll. 1983).

However, it is not known, whether changes in the function of the central nervous system (CNS) induced by such large doses can only be attributed to effects of chemotoxicity and hyperosmolality of the contrast medium solution. These changes might also, in part, be induced by dilution of the CSF with an attendant decrease in its cation concentrations.

It is known that an undisturbed functioning CNS requires a

relatively constant CSF composition. It has been shown that the cationic composition of the CSF is maintained within very narrow limits also when cation concentrations outside the blood-brain barrier (BBB) vary grossly (BRADBURY 1979, FISHMAN 1980). Experimentally induced changes in CSF cation composition cause electrophysiologic changes in the CNS and alter animal behaviour (BRADBURY 1979). It is therefore surprising that replacing most of rabbit CSF with iohexol 370 mg I/ml, which is 3.2 times hyperosmolal relative to the CSF and lacks all the inorganic and organic components of CSF, did not cause any behavioural changes in most of the animals (GOLMAN et coll. 1980, MALY et coll. 1983).

To date, nothing is known about the influence of molecular toxicity and/or hyperosmolality of non-ionic contrast media on the cation composition of the CSF.

The aim of the present investigation was to obtain information about the changes in concentrations of the four main CSF cations (Na, K, Ca, and Mg) that might occur following sub-arachnoid injection of two monomeric non-ionic contrast media with different neurotoxicity - iohexol and metrizamide - administered in solutions iso- and hyperosmolal relative to the CSF.

Material and Methods

Forty albino rabbits (Swedish Land), weighing 1.1 - 2.2 kg (median weight 1.25 kg), were anaesthetized with pentobarbital (Mebumal^R) 30 mg/kg. The anaesthetized animals were fixed with a belt around their pelvis in the right decubitus position on a table with the head end lowered 40° to insure the flow of hyperbaric contrast medium solution into the cranial subarachnoid space. The cisterna magna was punctured with a 25-gauge needle (Butterfly, ABBOT A.S.) and 0.3 ml of clear CSF was

sampled ("pre-injectional sample"). Thereafter, the contrast medium solution was injected with an infusion pump at a rate of 0.4 ml/min. Both contrast media - iohexol and metrizamide - were randomly injected in solutions iso- and hyperosmolal to the CSF at volumes that were adjusted so that the rabbits received the same molar amount of contrast medium per kg of body-weight (0.48 mmol/kg). Rabbits were randomly injected with iso-osmolal iohexol 144 mg I/ml, hyperosmolal iohexol 370 mg/I ml, iso-osmolal metrizamide 166 mg I/ml, and with hyperosmolal metrizamide 370 mg I/ml. The osmolality of these contrast media solutions, volumes injected per kg body-weight, weight of animals, and the number of animals in the four groups are presented in Table 1.

After withdrawing the needle, the distribution of contrast media within the subarachnoid space was checked with a lateral radiogram, and rabbits with a subdural deposition of contrast medium were excluded and replaced (12 rabbits). Thereafter, the rabbits were laid on a horizontal table with the head level with the spine. To preserve the hyperosmolality of the intracranial contrast medium bolus for as long a period as possible, mixing and dilution of the contrast medium bolus with the CSF as a result of movements of the animal were minimized by anaesthesia. Light anaesthesia, with spontaneous ventilation and preserved corneal reflex, was maintained for 3 hours with supplementary doses of pentobarbital. Three hours after the contrast medium injection, the cisterna magna was repunctured and 1-1.5 ml of the CSF was sampled for analysis ("final sample").

Both "pre-injection" and "final" CSF samples were analysed for their content of Na and K by flame photometry and their content of Ca and Mg by atomic absorption spectrometry. The iodine concentrations were determined by X-ray fluorescence analysis (GRÖNBERG et coll. 1983).

Table 1

Physical properties of injected solutions exposing the rabbits to equimolar doses (0.48 mmol) of contrast media per kg body-weight. Weight and number of rabbits within groups

Contrast medium	Concentration (mg I/ml)	Osmolality (mmol/kg H ₂ O)	Volume (ml/kg body-weight)	Median weight of animals (kg)	Number of animals
Iohexol*	144	300	1.28	1.30	9
Iohexol	370	980	0.50	1.20	13
Metrizamide*	166	300	1.11	1.25	9
Metrizamide	370	580	0.50	1.35	9

*Iso-osmolar to the CSF

Methodological considerations and calculations

Iohexol and metrizamide contain 0.1 and 0.2 mmol Ca per 100 mg iodine, respectively, as EDTA-Ca-Na. It was assumed that this EDTA-bound Ca proportionally follows the kinetics of the contrast medium within the subarachnoid space, and a concentration of this biologically inactive Ca, proportional to the contrast medium concentration in the final sample, was subtracted from the measured values of Ca in the final sample.

Thereafter, each measured concentration of the cation in "final sample" was expressed as a percentage of the "pre-injectional concentration" of the same cation in the same rabbit ($\text{Na}^{\frac{\circ}{2}}$, $\text{K}^{\frac{\circ}{2}}$, $\text{Ca}^{\frac{\circ}{2}}$, and $\text{Mg}^{\frac{\circ}{2}}$). By normalizing in this way, each rabbit served as its own control at the interpretation of cation shifts.

It can be assumed that the final sample of fluid from the subarachnoid space obtained from animals in this investigation represents a composite of the following four separate volume fractions:

1. The CSF volume fraction with the same cation composition as prior to the contrast medium injection - preprocedural CSF and newly formed CSF of "pre-injection composition".
2. The volume fraction of contrast medium solution with the concentration injected.
3. The volume fraction of water that has flowed into the subarachnoid space owing to the osmotic force of the hyperosmolar contrast media solutions.
4. The volume fractions of plasma and/or extracellular fluid of the body that may have "contaminated" the CSF if the permeability of the BBB was increased. Cation concentration of the "contaminating" volume fraction of plasma is equal to that of CSF as regards Na, and the concentrations of the three remaining cations in plasma, (K, ionized and protein-bound Ca,

and Mg) are higher than those in the CSF (DAVSON 1967). Thus, the volume fraction of plasma within the final sample, when based on its Na concentration, cannot be distinguished from the volume fraction of CSF and was therefore included in the term "partial volume of CSF" (see below).

In order to obtain information about the amount of contrast medium solution that remained in the subarachnoid space 3 hours after the contrast medium injection and to obtain information about a possible water inflow induced by the osmotic pressure of hyperosmolal solutions of iohexol and metrizamide, the volume fractions of CSF, contrast media solutions, and osmotically acquired water were calculated.

Na is the only cation that can be postulated to represent CSF, because by using any of the three remaining cations as a measure of the CSF, the volume fraction of the CSF would be overestimated if a volume fraction of plasma was present in the final sample. The volume fraction of the final sample, which contained all four cations in the same concentration as the pre-injectional CSF of the same rabbit - named as the "partial volume of CSF" (vCSF) - is given by the equation

$$vCSF = \frac{Na^{\%}}{100} \quad (1)$$

The volume fraction of the final sample, represented by the contrast medium solution - a partial volume of the contrast medium (vCM) - is given by the equation

$$vCM = \frac{\text{mg I/ml of the final sample}}{\text{mg I/ml of the contrast medium solution injected}} \quad (2)$$

The partial volume of osmotically acquired water in the final sample (v_{H_2O}) was postulated to be such a volume fraction of the final sample, which neither represented the volume fraction of CSF at its physiologic concentration (v_{CSF}) nor the volume fraction of contrast medium solution (v_{CM}) at the concentration injected:

$$v_{H_2O} = 1 - (v_{CSF} + v_{CM}) \quad (3)$$

The statistical evaluation of differences in calculated parameters was performed with the two-sided Wilcoxon rank sum test ($p < 0.05$ was considered to be a level of significance).

Results

The iso-osmolal solution of iohexol (144 mg I/ml) was the only one that caused a uniform decrease of all four cations from their pre-injectional levels (Table 2, Fig. 1). The same amount of iohexol molecules, when given in a hyperosmolal solution (370 mg I/ml), caused a significant increase of Ca^{++} above the level of the three remaining cations (Table 2, Fig. 1).

Contrary to the iso-osmolal solution of iohexol, the iso-osmolal solution of metrizamide (166 mg I/ml) caused a significant increase of both bivalent cations (Ca^{++} and Mg^{++}) above the level of both monovalent cations (Na^{+} and K^{+}) (Table 2, Fig. 1). The same number of metrizamide molecules, when given in a hyperosmolal solution (370 mg I/ml), similarly caused a significant increase of the two bivalent cations above the monovalent cations and, in addition, a significant increase of Ca^{++} above Mg^{++} (Table 2, Fig. 1).

The median concentration of Ca was higher than the pre-injectional Ca concentration after injection of both the iso-

osmolal and the hyperosmolal solution of metrizamide (Table 2, Fig. 1).

The concentration of contrast medium within the subarachnoid space ranged between 7 and 44 mg I/ml (median 16 mg I/ml) 3 hours after the contrast medium injection (Table 2, Fig. 2a).

There were no significant differences in iodine concentrations in the final CSF samples between groups of rabbits injected with different volumes and concentrations of both contrast media solutions. The iodine concentration (which expresses the concentration of contrast medium in mg I/ml) and the partial volume of contrast medium (v_{CM}) in the final sample were both weakly positively correlated with the animal weight ($r = 0.35$ and 0.27) (Fig. 2 a, b).

The calculation of water inflow showed that the hyperosmolal solutions of both contrast media caused significantly higher inflow of water into the subarachnoid space than their respective iso-osmolal preparations (Fig. 3). No significant differences in water inflow were found between iohexol and metrizamide when compared at the same osmolality level.

Table 2

Concentration of CSF cations in the final sample (3 h after subarachnoid injection of the contrast medium) expressed as a % of their pre-injectional concentration. (Median values of pre-injectional concentrations in mmol/l: Na = 156, K = 2.9, Ca = 2.3, Mg = 1.7). Concentration of contrast medium (given in mg I/ml of CSF), partial volume of contrast medium at its concentration in the solution injected, and partial volume of osmotically acquired water - all in the final sample. Median values: ranges within parentheses

Contrast medium	Iohexol		Metrizamide	
Solution injected (mg I/ml)	144	370	166	370
Dose (mmol contrast medium/kg body-weight)	0.48	0.48	0.48	0.48
Cations: Na ⁺	86 (77-98)	84 (73-98)	91 (81-99)	87 (77-94)
K ⁺	84 (76-97)	86 (72-118)	92 (70-106)	85 (78-124)
Ca ⁺	85 (72-123)	100 (76-144)	102 (77-131)	108 (82-135)
Mg ⁺	88 (75-113)	88 (67-113)	100 (75-115)	100 (88-100)
Contrast medium (mg I/ml of CSF)	15 (8-18)	15 (7-44)	16 (13-27)	18 (9-26)
Partial volume of contrast medium (x100)	10 (6-12)	4 (2-12)	10 (8-17)	5 (2-7)
Partial volume of water (x100)	5 (/ -12/-12)	13 (/ -1/-18)	1 (/ -6/-8)	8 (3-16)

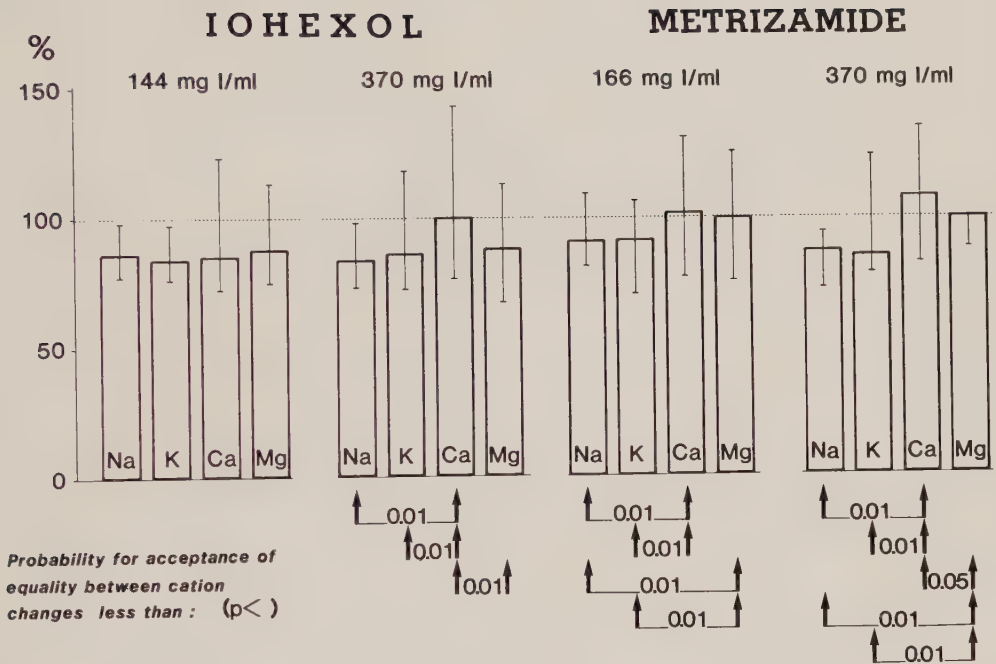
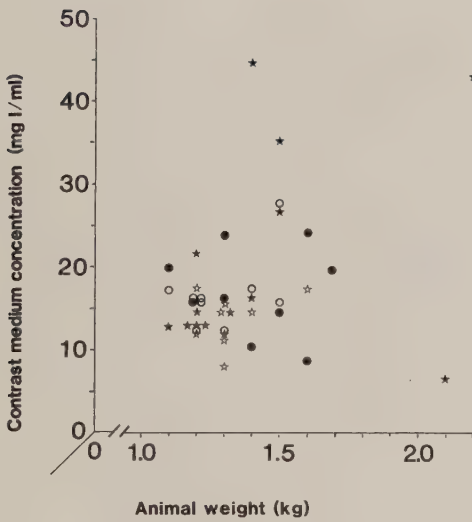
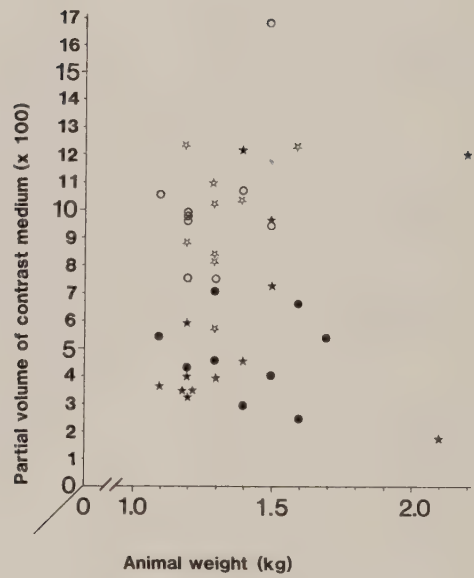


Fig. 1. Concentration of CSF cations in the final sample (3 h after subarachnoid injection of the contrast medium) expressed as a percentage of their pre-injectional concentration. Median and range. Probability for acceptance of equality between the percentual values of different cations within groups injected with the same contrast medium preparation (p -values).

a)



b)



*Iohexol 144 *Iohexol 370 ◐Metrizamide 166 •Metrizamide 370

Fig. 2. Scatter plot of contrast medium concentration (a) and partial volume of contrast medium (b) within the final sample versus animal weight.

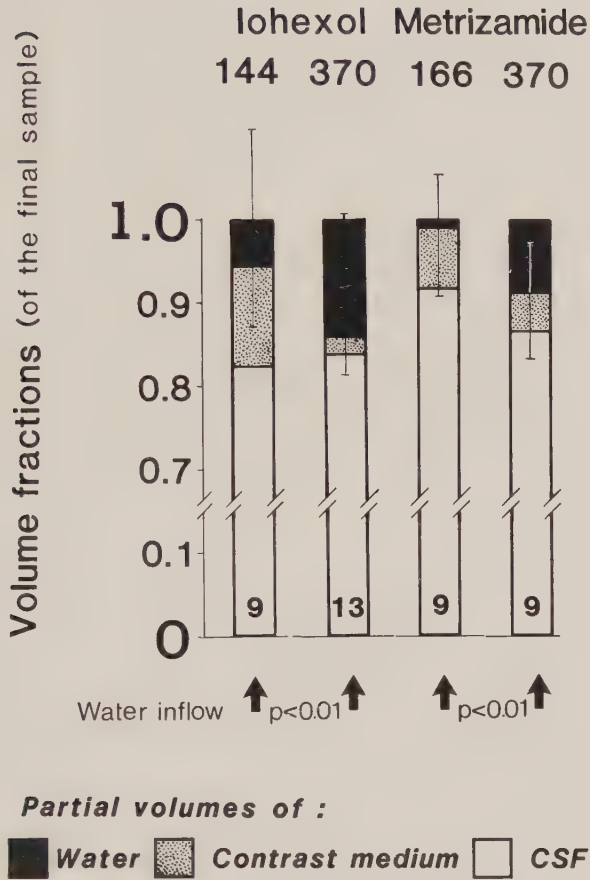


Fig. 3. Volume fractions (partial volumes) of CSF sample obtained 3 hours after injection of contrast medium into the subarachnoid space of anaesthetized rabbits. P-values of differences in partial volume of osmotically acquired water between groups injected with iso-osmolar (144 and 166 mg I/ml) and hyperosmolar (370 mg I/ml) solutions of contrast media.

Discussion

The absolute values of concentrations of iodine and CSF cations, presented under Results, might be influenced by several methodological artifacts - such as anaesthesia and possible leakage of CSF-contrast medium mixture through the puncture hole in the cisterna magna. The magnitude of the effects of these two artifacts on the rate of formation and the composition of the newly formed CSF was not determined. Such artifacts cannot, however, explain the obtained differences in cation concentrations and particularly not the increased CSF concentrations of Ca. Intermittent removal of CSF volume corresponding to the volume of newly formed CSF - in anaesthetized rabbits not injected subarachnoidally - has been shown (MALY et coll.) to cause a slight but significant decrease of Ca concentration in the CSF compared with concentrations of Na, K, and Mg.

As the rabbits used in the present investigation randomly underwent the same technical procedure, the influence of all the possible methodological artifacts is to be assumed to be the same in all the rabbits. The following discussion is therefore not based on the absolute values of the measured parameters, but exclusively on their relative changes within and between the four groups of experimental animals.

There is a theoretical possibility that the cation composition of the newly formed CSF could also be changed by some direct effect of contrast media on the mechanisms that control the exchange of cations on the BBB. However, no experimental mechanism has been reported that could increase the concentration of CSF Ca above its physiologic level.

The mechanism that could, in our opinion, explain the differences we found in CSF cation composition following subarachnoid injection of four different contrast medium solu-

tions is the contamination of the CSF by plasma or interstitial fluid of the body.

If the concentrations of all CSF cations are 100%, then, the concentration of Ca (both protein-bound and ionized) in the blood plasma is 226%, K 148%, and Mg 116%, whereas the concentration of Na is almost equal to that in the CSF (99%) (DAVSON 1967). If the CSF should become contaminated by plasma or its filtrate, the increase of Ca in the CSF would be the most conspicuous cation change. In such an event, an increase of K in the CSF, which theoretically may be expected to be the second largest one, might be reduced or eliminated by the powerful K-pump in the endothelial cells of brain capillaries, described by GOLDSTEIN (1979) and EISENBERG & SUDDITH (1979). Also, the theoretically expected increase of Mg in the CSF might be lowered by the homeostatic mechanisms because, as with K, also the control of Mg in CSF is exceedingly effective, whereas control of Ca in the CSF does not appear to be quite so effective (BRADBURY 1979, FISHMAN 1980). Thus, a contamination of the CSF by plasma could result in (1) a high increase in Ca; (2) at a higher degree of contamination, also a weak increase in Mg; and (3) no increase in Na or K. The degree of such a distribution of cation concentrations in the CSF may thus reflect the degree of the increase in BBB permeability caused by contrast medium solutions injected.

According to the degree of CSF cation changes in the final sample (Fig. 1), the contrast medium solutions injected in the present study can be ranked from the least to the most "BBB-damaging" solution as follows: Iohexol 144 mg I/ml; iohexol 370 mg I/ml; metrizamide 166 mg I/ml; metrizamide 370 mg I/ml.

Both chemotoxicity of contrast medium molecules and hyperosmolality of contrast medium solution have been demonstrated to increase the permeability of the BBB (HOPPE 1959, RAPOPORT 1973, RAPOPORT OCH LEVITAN 1974, STERRETT et coll. 1974,

IRSTAM et coll. 1982). Also decrease in CSF pH is considered to be involved in the mechanisms altering the BBB permeability (FISHMAN 1980). A deficient buffering capacity of contrast medium injected into the subarachnoid space may lead to a decrease in CSF pH, analogously as in experiments of ELLIOTT and JASPER (1949). Of the contrast media used in the present investigation, iohexol 370 mg I/ml has a high buffer capacity and contains 10 mmol Tris buffer, whereas the buffer capacity of metrizamide 370 mg I/ml is very small, as it contains only 0.6 mmol of HCO_3 .

Hypothetically, the contrast medium solutions injected in the present investigation can also be ranked according to combinations of their characteristics - molecular toxicity, hyperosmolality and buffering capacity - each of which is able to mediate the opening of the BBB. Their hypothetical rank order from the least to the most BBB damaging solution would be: Iohexol 144 mg I/ml - low chemotoxicity, well buffered and iso-osmolal; iohexol 370 mg I/ml - the same low chemotoxicity as the former, well buffered but with 3.2 times higher osmolality than the CSF; metrizamide 166 mg I/ml - higher chemotoxicity than iohexol, lacking buffer and iso-osmolal; metrizamide 370 mg I/ml - the same higher chemotoxicity as the preceding, even lacking buffer but hyperosmolal.

The agreement between the obtained and the hypothetical rank orders of contrast medium solutions support the hypothesis that changes in CSF cation concentrations, observed in the present investigation, could be explained by increased permeability of the BBB.

In the present investigation, subarachnoidally injected metrizamide caused a greater increase than iohexol of Ca and Mg over Na and K in the CSF. Metrizamide has also induced significantly more frequent and severe depressive changes in the behaviour of non-anaesthetized rabbits than iohexol

(GOLMAN et coll. 1980, MALY et coll. 1983). Experimentally increased concentrations of Ca and Mg in the CSF are known to have a depressant effect on animal behaviour (BRADBURY 1979). Therefore, it is theoretically possible that the depressive effects of subarachnoidally injected contrast media on animal behaviour may, in part, be mediated by an increase of CSF concentrations of Ca and Mg relative to those of Na and K.

With the aim of exposing the CNS of the animals with different body-weights to a quantitatively similar effect of contrast medium, two modes of dosage are employed in contrast medium research. The contrast medium is administered subarachnoidally either at a dose proportional to the animal weight or at an equal dose to all animals regardless of their weights. The weight of the brain and the spinal cord have both been found to be linearly correlated with the body-weight and length of the rabbit (LATIMER & SAWIN 1955), but no data are available stating the correlation between the volume of the subarachnoid space and body-weight of the rabbit.

In the present investigation, doses of contrast medium were given in direct proportion to animal weight. If the volume of the subarachnoid space of the rabbit was not positively correlated to the animal weight, then a strong correlation between the animal weight and the contrast medium concentration in the final sample was to be expected. However, in the present investigation only very weak positive correlations between body-weight and both the contrast medium concentration and the partial volume were obtained ($r = 0.35$ and 0.27). This indicates that no major systematic error is made when a contrast medium is injected into the subarachnoid space at doses directly proportional to the animal's weight.

Conclusions

CSF cation changes following subarachnoid injection of contrast media deserve attention as they may participate in the total neurotoxic effect of contrast medium.

Depressive effects of subarachnoidally injected contrast media on animal behaviour may, in part, be mediated by increases in Ca and Mg concentrations in the CSF. Such a change in CSF cation composition may follow a contrast medium-induced increase in blood-brain barrier permeability.

REFERENCES

- BRADBURY M.: The concept of a blood-brain barrier. Ed.: John Wiley & Sons, Chichester, New York, Brisbane, Toronto 1979.
- DAVSON H.: Physiology of the cerebrospinal fluid. Ed.: J & A Churchill, London 1967.
- EISENBERG H.M. and SUDDITH R.L.: Cerebral vessels have the capacity to transport sodium and potassium. Science 206 (1979), 1083.
- ELLIOTT K.A.C. and JASPER H.H.: Physiological salt solutions for brain surgery. Studies of local pH and pial vessel reactions to buffered and unbuffered isotonic solutions. J. Neurosurg 6. (1949), 140.
- FISHMAN R.F.: Cerebrospinal fluid in diseases of the nervous system. Ed.: W.B. Saunders Co., Philadelphia, London, Toronto 1980.
- GOLDSTEIN G.W.: Relation of potassium transport to oxidative metabolism in isolated brain capillaries. J. Physiol. (London) 286 (1979), 185.
- GOLMAN K., OLIVECRONA H., GUSTAFSON C., SALVESEN S., ALMÉN T. and MALY P.: Excitation and depression of non-anaesthetized rabbits following injection of contrast media into the subarachnoid space. Acta Radiol. /Suppl/ (Stockh) 362 (1980), 83.
- GONSETTE RE.: Immediate and delayed neurotoxicity of newly synthesized contrast media demonstrated by EEG sequential analysis. In: Amiel M., ed. Contrast media in radiology. p. 141. Springer-Verlag, Berlin Heidelberg New York 1982.

GRÖNBERG T., SJÖBERG S., GOLMAN K. and MATTSSON S.: Non-invasive estimation of kidney function by X-ray fluorescence analysis: Elimination rate and clearance of contrast media in man. Accepted for publication in Invest. Radiol. 1983.

HOPPE J.O.: Some pharmacological aspects of radiopaque compounds. Ann. NY Acad. Sci. 78 (1959), 727.

IRSTAM L., PERSSON L.I., VÄLLFORS B., HANSSON E., BELGHMAIDI M., RÖNNBÄCK L. and HANSSON H-A.: Neurotoxicitet hos röntgenkontrastmedel. (In Swedish). Acta Societatis Medicorum Suecanae Hygiea 91 (1982), 198.

LATIMER H.B. and SAWIN P.B.: The weight of the brain, of its parts and the weight and length of the spinal cord in the rabbit (race x). J. Comp. Neurol. 103 (1955), 513.

MALY P., OLIVECRONA H., ALMÉN T. and GOLMAN K.: Interaction between chlorpromazine and intrathecally injected non-ionic contrast media in non-anaesthetized rabbits. Submitted to Neuroradiology 1983.

MALY P., ALMÉN T., FEX G., GOLMAN K. and OLIVECRONA H.: Course of CSF cation changes after subarachnoid injection of non-ionic contrast media in rabbit. Manuscript.

RAPOPORT S.I. and LEVITAN H.: Neurotoxicity of X-ray contrast media. Relation to lipid solubility and blood-brain barrier permeability. AJR 122 (1974), 186.

RAPOPORT S.I.: Reversible opening of the blood-brain barrier by osmotic shrinkage of the cerebrovascular endothelium. In: Hilal S.K., ed. Small vessel angiography, p. 137. C.V. Mosby Co., Saint Louis 1973.

STERRETT P.R., THOMPSON A.M., CHAPMAN A.L. and MATZKE H.A.:

The effects of hyperosmolarity on the blood-brain barrier. A morphological and physiological correlation. Brain Res. 77 (1974), 281.



